JCI The Journal of Clinical Investigation

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J Clin Invest. 1969;48(10):1809-1819. https://doi.org/10.1172/JCI106147.

Research Article

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The apparent "MCR'S" calculated on the basis of the 2-hr studies expressed as liters/24 hr per m^2 ±SD were: "MCR^{E_1}" (women) 980 ±94, (men) 1170 ±95; "MCR^{E_2}" (women) 615 ±17, (men) 830 ±30. The estradiol "MCR's" differed significantly between men and women. "MCR^{E_2}" was the same using either estradiol-^{14}C or -^{3}H and was unchanged by the infusion of 170 μg of estradiol daily. Postmenopausal women had estrogen "MCR's" in the same range as premenopausal women. Excess glucocorticoids increased the "MCR^{E_2}."

A Study of Estrogen Metabolic Clearance Rates and Transfer Factors

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ABSTRACT We have attempted to measure the metabolic clearance rates (MCR) and the transfer factors of estradiol (E_2) and estrone (E_1) during 2-hr and 12-hr infusions. When estradiol-^{3}H was infused for 2 hr, apparent equilibrium was reached at 70 min; the 12-hr infusions showed that plasma estradiol-^{3}H levels increased slowly throughout the infusion. When estrone-^{3}H was infused, constancy of estrone-^{3}H levels was not attained in either the 2-hr infusions or in the two 12-hr infusions. The tritium level in the metabolite of the infused estrogen did not become constant in 50% of the short infusions and increased during all the long infusions. Thus, the conversion ratios $C^{E_1E_2}$ and $C^{E_2E_1}$ continually changed and transfer factors could not be calculated.

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INTRODUCTION

The concepts of metabolic clearance rate (1) and transfer factors for interconverting compounds (2, 3) have proved useful in the study of androgen metabolism (4, 5). We have utilized the technique of constant infusion of radioactive tracers to study the clearance rates of estrone and estradiol and to estimate the extent of net interconversion or transfer factors. Although the preliminary results (6) were in substantial agreement with those of Longcope, Layne, and Tait (7), our data suggested that constant levels of isotope were not always attained during these infusions. Since estimates of these parameters can be meaningful only when the steady-state requirement is met, we have examined in more detail the kinetics of estrone-estradiol metabolism. The effect of such variables as mass of estrogen infused, length of infusion, and hormonal status on the metabolic clearance rate (MCR) was investigated.

METHODS

Materials. Estrone-6-7-^{3}H, estradiol-17β-6,7-^{3}H (34c/mmole) (New England Nuclear Corp.), and estrone-4-^{14}C (29 mc/mmole) and estradiol-17β-4-^{14}C (53.7 mc/mmole) (Nuclear-Chicago) were purified by thin-layer chromatography (TLC) and stored at 4°C. Aliquots of ^{3}H- and ^{14}C-labeled steroids were combined and radiochemical purity was demonstrated before use by constancy of the ^{3}H/^{14}C ratio through a series of derivatives (Fig. 1). Steroids obtained from Mann Research Labs Inc. were recrystalized before use in the reverse-isotope dilution studies. Solvents used for chromatography were redistilled before use and chemical reagents were used without further purification.

Chromatography. Merck precoated Silica Gel GF-254 20 × 20 cm glass plates were used for TLC. The systems used for each compound and the R_f values are given in Table I.

Gas-liquid chromatography was performed with a Glowall Corporation model No. 310 using 3% SE-30 packing (Applied Science Laboratories Inc.) and a hydrogen flame detector. The height of the peak response was a linear function of the mass injected between 20 and 140 ng and 2 SE ranged between 2 and 3%.

Subjects. Normal subjects (Nos. 1–22), ages 21–30 yr, were hospitalized at the Clinical Center of the National Institutes of Health, and received no medications. Each woman had normal cyclic menses. The three normal postmenopausal women (Nos. 23, 24, and 26) were aged 52, 53, and 67 yr, respectively, and received no medications. Patients (Nos. 25, 27, 28, 32, and 34) had metastatic carcinoma of the breast with normal kidney, hepatic, bone marrow, and endocrine function at the time of the study. A 19 yr old man (No. 33) with metastatic choriocarcinoma, had received no therapy when studied. The effect of excess glucocorticoids on estrogen metabolic clearance rates was examined in the following patients: a 54 yr old postmenopausal woman (No. 29) with severe Cushing's syndrome of 3 months' duration (plasma cortisol 40–65 μg/100 ml) associated with an undifferentiated carcinoma of the lung;

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Received for publication 14 February 1969 and in revised form 6 June 1969.

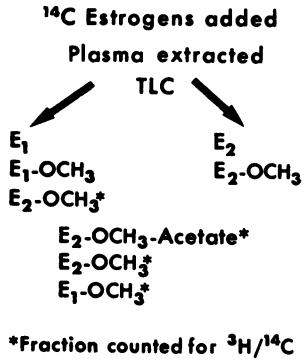

FIGURE 1 Outline of the procedures used to establish radiochemical purity of plasma steroids.

a 55 yr old woman (No. 30), with carcinoma of the breast metastatic to lung and bone, who received 100–150 mg cortisone acetate daily after bilateral adrenalectomy for 5 days before study; and a 39 yr old woman (No. 25) with carcinoma of the breast metastatic to bone, who was given 40 U adrenocorticotropic hormone (ACTH) intramuscularly for 10 days and estradiol-^{3}H infused on day 4 and day 9. The effect of the androgen, fluoxymesterone, was observed in patient No. 31, a 27 yr old infertile male with increased estrogen production and a plasma testosterone level of 0.25 μg/100 ml, who was studied before and during administration of fluoxymesterone 10 mg/day for 5 days.

Clearance rates. Short infusions were performed between 6 a.m. and 9 a.m. with the subjects in the basal state. The metabolic clearance rates (MCR's) were performed as described previously (5). The total dose of radioactivity given was 30–40 μc of tritium or 4–5 μc of carbon-14. One-third of the total dose was given over a period of 2–3 min as a priming dose 30 min before the infusion was begun. 30–40 ml of blood was drawn in heparinized syringes at 50, 70, 90, and 120 min after starting the infusion; the plasma was separated immediately by centrifugation and was stored at −16°C until extracted.

In subject No. 34 the effect of intravenous administration of estradiol (E_2) upon MCR^{E_2} was evaluated. A sterile stock solution of estradiol (10 mg/ml) in propylene glycol was prepared by the pharmacy. The steroid was dissolved in ethanol and added to isotonic saline (to give a 5% ethanol solution). Estradiol was given continuously by infusion pump for 6 days. Each of three dose levels (17, 170, and 1440 μg/day) was infused for 48 hr. The MCR^{E_2} was determined before, at the end of each dose, and the day after the 6 day infusion by a 2 hr infusion of E_2-^{3}H. Assuming no endogenous estrogen production, the plasma estrogen levels were calculated from the rate of estrogen infusion and the plasma estrogen radioactivity during the MCR study. The specific activity of each estradiol infusion was checked by gas-liquid chromatography and tritium measurement of ether extracts of the infusate and agreed well with the calculated specific activities.

Long infusions. In four subjects (Nos. 27, 28, 32, and 33) the infusion period was 8–12 hr. These subjects were given a total dose of 200–400 μc. Only patient No. 28 received a priming dose of 10 μc 30 min before the start of the infusion. Blood samples were obtained at hourly intervals, or more frequently, throughout the infusion period and patients remained supine except to use a bed pan. During the infusions patients were given a snack of tea and toast.

Extraction and derivative formation. 25 ml water and 150–200 μg of estrone (E_1) and estradiol (E_2) were added to each plasma sample. To samples containing only ^{3}H-labeled steroids, 100–400 cpm of estradiol-4-^{14}C and estrone-4-^{14}C were added to correct for losses. In samples containing both ^{3}H- and ^{14}C-labeled steroids, correction for procedural losses was made by reverse isotope dilution using gas-liquid chromatography to measure estrone and estradiol derivatives. All plasma samples were extracted three times with two volumes of ether. The extracts were chromatographed by two-dimensional TLC by developing each plate first with 100% petroleum ether, then twice in system I and finally at right angles twice in system V. This procedure resulted in sharp zones opposite the reference standards which were easily visible under ultraviolet light and which were free of contaminating lipid or pigment. Recoveries were 80–95%.

TABLE I
Thin-Layer Systems

Steroids	I Benzene 70: ethyl acetate 30	II Benzene 80: ethyl acetate 20	III Benzene 90: ethyl acetate 10	IV Benzene 90: MeOH 10	V Methylene chloride 80: ethyl acetate 20
Estrone	0.60*	0.60	0.15	—	0.60
Estradiol	0.45	0.30	—	—	0.40
Estrone-3-methyl ether	—	0.80	0.45	0.20	—
Estradiol-3-methyl ether	—	0.55	0.20	0.10	—
Estradiol-3-methyl ether-17β-acetate	—	—	—	0.45	—

* Relative mobility.

Methylation was performed by a modification of Brown's procedure (8). Samples were dried in 100-ml conical tubes, dissolved in 0.1 ml ethanol plus 25 ml of a 2.0% boric acid solution, and brought to pH 11.2 with 20% NaOH. After addition of 0.5 ml dimethyl sulfate, tubes were shaken vigorously and incubated at 37°C for 30 min. The incubation was repeated after the addition of 0.5 ml dimethyl sulfate and 1.0 ml 20% NaOH. Excess dimethyl sulfate was neutralized with 5.0 ml 20% NaOH and the reaction mixture was extracted with 50 ml petroleum ether, the organic phase transferred to a 50 ml conical tube and washed two times with 2 ml 0.1 M acetic acid. The yield after TLC of estrone-3-methyl ether was 85–95% and of 17β-estradiol-3-methyl ether was 80–90%. Estrone-3-methyl ether was reduced with sodium borohydride. 17β-estradiol-3-methyl ethers were acetylated and oxidized with a partially purified 17-ol-dehydrogenase (9). The yields of each derivative were 80–90%. Fig. 1 outlines the procedures followed to establish radiochemical purity of each plasma steroid. Radioactivity was measured in a Packard Tri-Carb liquid scintillation spectrometer, model 574, at a tritium efficiency of 27% and carbon-14 efficiency of 57%. 18% of the carbon-14 counts were counted in the tritium channel.

Statistics and calculations. The 95% confidence limits of each ^{3}H/^{14}C ratio were calculated as described in the Appendix. When the ratios of the three derivatives did not differ significantly, the average was used in subsequent calculations. Otherwise the lowest ^{3}H/^{14}C ratio was assumed to be the final one. In 89% of the samples, ^{3}H/^{14}C did not change after the first derivative, in 6% the last two ^{3}H/^{14}C ratios were the same and lower than the first, and in 2% the last ^{3}H/^{14}C of the third

TABLE II

Plasma Tritium Levels and "Metabolic Clearance Rates" in Normal Men (Short Infusions)

Subject	Body surface area	Rx estrone-^{3}H infusion rate	Estrone (X^{E_1}) 50 min	70 min	90 min	120 min	Mean	MCR^{E_1}	MCR^{E_1}/m^2	Estradiol (X^{E_2}) ±95% confidence limits 50 min	70 min	90 min	120 min
	m^2	*dpm/hr*	*dpm/liter × 10^{-5}*					*liters/24 hr*	*liters/24 hr per m^2*	*dpm/liter × 10^{-4}*			
1	2.00	2.07×10^7	2.73	2.92		3.06	2.90	1713	857		0.78 ±0.06		1.45 ±0.14
2	2.10	2.18×10^7	2.39	2.24		2.45	2.36	2216	1056		1.03 ±0.08	1.07 ±0.10	1.34 ±0.14
3	2.13	6.76×10^7	6.24	6.33	6.76	6.76	6.52	2487	1168	3.48 ±0.24	4.32 ±0.28	4.15 ±0.22	5.82 ±0.30
4	2.30	4.96×10^7	3.28	3.27	3.75	3.72	3.50	3400	1400	1.37 ±0.12	1.18 ±0.08	2.00 ±0.10	2.10 ±0.15
5	1.75	2.00×10^7	2.02	2.33	2.21	2.33	2.23	2152	1230	1.26 ±0.06	1.13 ±0.07	1.35 ±0.07	1.95 ±0.10
Mean ±SEM								2412 ±258	1168 ±95				
		Rz estradiol-^{3}H infusion rate	Estradiol (z^{E_2}) 50 min	70 min	90 min	120 min	Mean	MCR^{E_2}	MCR^{E_2}/m^2	Estrone (z^{E_1}) ±95% confidence limits 50 min	70 min	90 min	120 min
6	2.00	2.28×10^7		3.63		3.53	3.58	1528	764		2.50 ±0.23		3.30 ±0.26
7	1.90	2.08×10^7		3.74		3.27	3.51	1422	748		3.15 ±0.32		2.75 ±0.22
8	2.13	4.18×10^7	4.58	4.96	5.89	6.05	5.37	1868	849	4.75 ±0.29	8.95 ±0.68	9.15 ±0.73	8.87 ±0.97
9	2.30	4.11×10^7	4.57	4.66	5.01	4.96	4.80	2055	893	4.68 ±0.47	3.28 ±0.39	5.28 ±0.42	6.42 ±0.32
10	1.52	2.49×10^7	4.34	4.44	4.07	5.17	4.51	1325	883	3.00 ±0.21	3.79 ±0.30	3.80 ±0.40	4.79 ±0.40
Mean ±SEM								1640 ±139	827 ±30				

derivative was the highest. A fourth derivative confirmed the ratio of the third in each case.

The 95% confidence limits of plasma ^{3}H or ^{14}C in the reverse isotope dilution studies were obtained by first assessing the error of the isotope dilution procedure. When 10 samples of identical specific activity were processed as in Fig. 1, 95% confidence limits were ±6%.

The symbols and other calculation are those used by Longcope et al. (7); z refers to the isotope in E_2, x to the isotope in E_1, superscripts to the steroids, and subscripts to the compartments. The formulas used are as follows:

$$MCR^{E_1} = Rx^{E_1}/x^{E_1} \quad \text{and} \quad MCR^{E_2} = R_3^{E_2}/z^{E_2}$$

where Rx^{E_1} and $R_3^{E_2}$ are the infusion rates expressed as dis-

TABLE III

Plasma Tritium Levels and "Metabolic Clearance Rates" in Normal Women (Short Infusions)

Subject	Body surface area	Rx estrone-^{3}H infusion rate	Estrone (X^{E_1}) 50 min	70 min	90 min	120 min	Mean	MCR^{E_1}	MCR^{E_1}/m^2	Estradiol (X^{E_2}) ±95% confidence limits 50 min	70 min	90 min	120 min
	m^2	*dpm/hr*	*dpm/liter × 10^{-5}*					*liters/24 hr*	*liters/24 hr per m^2*	*dpm/liter × 10^{-4}*			
11	1.76	2.40×10^7	3.27	3.78	3.61	3.81	3.62	1591	904	2.75 ±0.22	2.52 ±0.13	2.46 ±0.22	2.50 ±0.23
12	1.75	1.38×10^7	1.35	1.28	1.30	1.55	1.37	2417	1381	0.56 ±0.05	0.82 ±0.08	0.80 ±0.06	0.87 ±0.09
13	1.67	1.81×10^7	2.50	2.76	2.37	2.55	2.54	1710	1024	1.28 ±0.06	1.64 ±0.08	1.47 ±0.12	2.14 ±0.15
14	1.67	1.51×10^7			2.65	2.84	2.75	1318	789	1.14 ±0.09	1.45 ±0.09	1.56 ±0.14	3.09 ±0.28
15	1.47	1.64×10^7	3.70	3.44	3.65	3.51	3.58	1100	748	2.29 ±0.03	2.30 ±0.03	2.87 ±0.13	2.32 ±0.08
16	1.60	1.28×10^7	1.77	1.64	1.87	2.06	1.84	1670	1043	1.96 ±0.12	3.29 ±0.20	3.19 ±0.16	3.00 ±0.18
Mean ±SEM								1634 ±183	982 ±94				
		Rz estradiol-^{3}H infusion rate	Estradiol (z^{E_2}) 50 min	70 min	90 min	120 min	Mean	MCR^{E_2}	MCR^{E_2}/m^2	Estrone (z^{E_1}) ±95% confidence limits 50 min	70 min	90 min	120 min
17	1.76	1.88×10^7	3.23	4.22	4.06	3.74	4.01	1123	638	2.91 ±0.35	3.64 ±0.22	4.40 ±0.26	3.76 ±0.30
18	1.75	1.42×10^7	2.88	3.25	3.25	3.26	3.25	1049	596	2.70 ±0.22	3.86 ±0.25	4.40 ±0.37	7.13 ±0.74
19	1.67	1.91×10^7	4.02	4.23	3.40	4.51	4.04	1134	679	4.10 ±0.25	4.52 ±0.23	4.80 ±0.19	4.83 ±0.26
20	1.67	1.73×10^7	3.55	4.40	4.65	4.29	4.45	934	560	3.30 ±0.17		6.30 ±0.70	5.52 ±0.44
21	1.55	1.66×10^7	4.03	4.24	4.93	4.27	4.37	912	588	4.41 ±0.22	5.22 ±0.21	4.78 ±0.24	7.07 ±0.42
22	1.60	1.28×10^7	2.83	2.99	3.47	3.01	2.08	997	623	1.44 ±0.07	1.48 ±0.07	1.78 ±0.09	2.67 ±0.11
Mean ±SEM								1025 ±38	614 ±17				

TABLE IV

Plasma Tritium Levels and Conversion Fractions

Subject		Infusion rate	Time from start of infusion: 30 min	60 min	90 min	120 min	3 hr	4 hr
		dpm/hr						
Estrone								
32	*X^{E_1}	5.10×10^7	4.92 ±0.10	6.66 ±0.07	6.51 ±0.08	6.07 ±0.12	7.10 ±0.07	8.02 ±0.08
	X^{E_2}		1.45 ±0.09	1.67 ±0.10	2.05 ±0.10	2.75 ±0.11	3.11 ±0.12	3.54 ±0.12
	‡$\frac{X^{E_2}}{X^{E_1}}$		2.9 ±0.2	2.5 ±0.2	3.1 ±0.2	4.5 ±0.2	4.4 ±0.2	4.4 ±0.2
33	X^{E_1}	3.59×10^7		2.29 ±0.07	3.40 ±0.10	3.50 ±0.10	3.30 ±0.10	
	X^{E_2}			0.97 ±0.06	1.96 ±0.08	2.60 ±0.13	2.44 ±0.12	2.34 ±0.14
	$\frac{X^{E_2}}{X^{E_1}}$			4.2 ±0.3	5.8 ±0.3	7.4 ±0.4	7.4 ±0.4	
Estradiol								
27	§z^{E_2}	7.19×10^7		1.84 ±0.05		1.90 ±0.05	2.09 ±0.05	2.14 ±0.05
	z^{E_1}			0.59 ±0.04		1.10 ±0.07	1.67 ±0.10	2.27 ±0.09
	$\frac{z^{E_1}}{z^{E_2}}$			3.2 ±0.2		5.8 ±0.4	8.0 ±0.5	10.6 ±0.5
28	z^{E_2}	7.81×10^7						
	z^{E_1}			0.91 ±0.05		1.06 ±0.06	1.43 ±0.05	1.65 ±0.06
	$\frac{z^{E_1}}{z^{E_2}}$			(4.5)		(5.2)	(7.1)	(8.2)

* X^{E_1} represents tritium levels in estrone (dpm/liter $\times 10^{-6}$) ±95% confidence limits; X^{E_2} represents tritium levels in estradiol (dpm/liter $\times 10^{-5}$) ±95% confidence limits.

‡ Conversion fractions, $\frac{X^{E_2}}{X^{E_1}}$ and $\frac{z^{E_1}}{z^{E_2}}$ ($\times$ 100) ±95% confidence limits.

§ z^{E_2} represents tritium levels in estradiol (dpm/liter $\times 10^{-6}$) ±95% confidence limits; z^{E_1} represents tritium levels in estrone (dpm/liter $\times 10^{-5}$) ±95% confidence limits. Assumed conversion fraction based on the assumption that plasma tritium levels at 60 min. through 4 hr were 1.95×10^6 dpm/liter.

integration per minute per hour and x^{E_1} and z^{E_2} as disintegration per minute per liter of plasma. The value for MCR is expressed as liters per day. The conversion fractions are calculated from plasma isotope levels as follows:

$$C_{BB}{}^{E_2E_1} = z^{E_1}/z^{E_2} \quad \text{and} \quad C_{BB}{}^{E_1E_2} = X^{E_2}/X^{E_1}.$$

We have assumed that the variance of individual plasma isotope levels is equal to the variance of the respective $^3H/^{14}C$ ratios since the error associated with the measurement of the marker steroid radioactivity is small. The 95% confidence limits for individual conversion fractions at single points in time can therefore be calculated as the confidence limits for the ratio of two independent ratios (Appendix I). The 95% confidence limits of a conversion fraction at equilibrium where mean X^{E_1} and X^{E_2} and their variances are computed from isotope levels in multiple plasma samples can be determined as in Appendix II. The transfer factor, $[\rho]$, is calculated as follows:

$$[\rho]_{BB}{}^{E_1E_2} = C_{BB}{}^{E_1E_2} \times \frac{MCR^{E_2}}{MCR^{E_1}};$$

$$[\rho]_{BB}{}^{E_2E_1} = C_{BB}{}^{E_2E_1} \times \frac{MCR^{E_1}}{MCR^{E_2}}.$$

RESULTS

2-Hr infusions. Plasma tritium levels in the infused steroid and in its metabolite are given in Tables II and III. The relative change in plasma tritium levels during the infusions was evaluated by assigning the tritium level at 50 min a value of 1.00.

In the estradiol-^3H infusions, mean plasma estradiol levels (±SEM) were 1.11 ±0.03 at 70 min, 1.15 ±0.05 at 90 min, and 1.15 ±0.03 at 120 min. There was no significant trend to these values, but all were significantly higher than the 50 min value. In the estrone-^3H infusions, mean plasma tritium levels in estrone (±SEM) were 1.03 ±0.03 at 70 min, 1.05 ±0.02 at 90 min, and 1.10 ±0.03 at 120 min.

In contrast to the estradiol studies, group analysis of the estrone data suggested an upward trend of the ratios although only the 120 min value was significantly different from 1.00.

Although we recognized that constant tritium levels were not attained during all the estrone infusions, the

During 12-hr Infusions of Estrone and Estradiol

Time from start of infusion							
5 hr	6 hr	7 hr	8 hr	9 hr	10 hr	11 hr	12 hr
7.91 ±0.08	8.10 ±0.08	8.18 ±0.08	9.51 ±0.08	9.39 ±0.09	9.48 ±0.09	8.62 ±0.08	8.80 ±0.08
4.30 ±0.13	4.72 ±0.14	4.91 ±0.12	5.54 ±0.12	5.63 ±0.12	7.42 ±0.22	7.15 ±0.21	7.88 ±0.24
5.5 ±0.2	5.8 ±0.2	6.0 ±0.2	5.8 ±0.1	6.0 ±0.1	7.8 ±0.2	8.3 ±0.2	8.9 ±0.3
4.20 ±0.13	3.98 ±0.12	3.98 ±0.08	4.20 ±0.08	4.13 ±0.08	4.11 ±0.08	4.86 ±0.08	4.54 ±0.07
3.09 ±0.19	2.83 ±0.17	3.22 ±0.12	2.95 ±0.11	3.54 ±0.12	3.46 ±0.12	3.50 ±0.11	3.67 ±0.11
7.4 ±0.5	7.1 ±0.5	8.1 ±0.3	7.0 ±0.3	8.6 ±0.3	8.4 ±0.3	7.4 ±0.3	8.1 ±0.2
2.19 ±0.04	2.11 ±0.05	2.16 ±0.04	2.32 ±0.04	2.29 ±0.04	2.20 ±0.04	2.26 ±0.05	2.39 ±0.04
2.32 ±0.09	2.58 ±0.10	3.28 ±0.16	3.59 ±0.11	3.56 ±0.11	3.49 ±0.10	3.71 ±0.11	4.27 ±0.11
10.6 ±0.4	12.2 ±0.6	15.2 ±0.8	15.5 ±0.5	15.5 ±0.6	15.9 ±0.8	16.4 ±0.6	17.9 ±0.7
1.95 ±0.02	1.96 ±0.02	2.09 ±0.02	2.09 ±0.02				
1.69 ±0.06	1.92 ±0.09	1.85 ±0.08	1.95 ±0.08				
8.7 ±0.5	9.8 ±0.5	8.9 ±0.4	9.3 ±0.4				

apparent MCR^{E_1} was calculated from the mean plasma tritium levels for each study. MCR^{E_1} was 2410 ±258 liters/24 hr in men and 1630 ±183 liters/24 hr in women (P 0.05). After correction for body surface area, MCR^{E_1} was 1170 ±95 liters/24 hr per m^2 in men and 980 ±94 liters/24 hr per m^2 in women, the difference not being significant ($P > 0.10$). The apparent MCR^{E_2} was 1640 ±139 liters/24 hr in men and 1025 ±38 liters/24 hr in women (P 0.01). The corrected MCR^{E_2} of men (830 ±30 liters/24 hr per m^2) remained significantly different (P 0.001) from that of women (615 ±17 liters/24 hr per m^2).

When the tritium in the metabolite of the infused estrogen was examined, it could be seen that tritium levels did not become constant in all studies. In 10 of the 17 estrone infusions, constant tritium levels in estradiol were not attained (Tables II and III and Fig. 2). When the data from the entire group were analyzed, plasma tritium levels in estradiol during estrone-^{3}H infusions were 1.19 ±0.09 at 70 min, 1.27 ±0.08 at 90 min, and 1.57 ±0.17 at 120 min taking the 50 min value as 1.00. Similarly, mean plasma tritium levels in estrone during estradiol-^{3}H infusions were 1.22 ±0.12, 1.43 ±0.11, and 1.66 ±0.14 at 70, 90, and 120 min, respectively (Table II, Fig. 2). Mean values at 90 and 120 min were different from 1.00 in both groups and a significant trend was indicated. In 6 of the 12 estradiol infusions, constant tritium levels in plasma estrone levels were not reached (Tables II and III, Fig. 2).

Long infusions. The data from the four studies are presented in Table IV. Only in patient No. 28 was a priming dose of 10 μc given 30 min before the infusion. When estrone-^{3}H was infused, constancy of plasma ^{3}H levels in estrone was not maintained, although for 2- to 3-hr periods, there was apparent constancy of the isotope concentration (Fig. 3). The tritium levels in the metabolite, estradiol, increased for 12 hr in patient No. 32 and for 9 hr in patient No. 33. In addition, the conversion ratios showed the same trend.

When estradiol-^{3}H was infused (Fig. 4) there was a small but significant increase in plasma estradiol-^{3}H

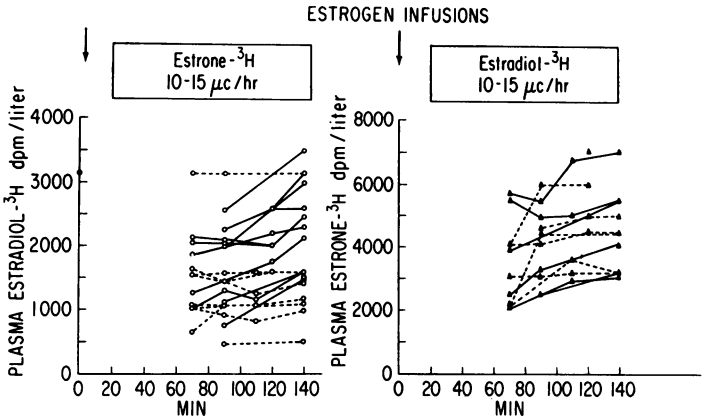

FIGURE 2 Plasma tritium levels in product steroids, estradiol (left) and estrone (right), during infusions of precursors estrone-^{3}H and estradiol-^{3}H. The studies in which the plasma tritium levels did not become constant are indicated by the solid lines and those in which tritium did become constant by the broken lines.

levels. However, the tritium in plasma estrone increased throughout the infusion so that in patient No. 27, the conversion ratio varied from 3.2 at 1 hr to 8.9 at 12 hr.

Since measurement of a metabolic clearance rate requires that the plasma level of the infused isotope reach equilibrium, a true MCR for estrone and estradiol cannot be obtained by these techniques. We have, therefore, elected to place quotes about the term "metabolic clearance rate" to distinguish the measurements obtained herein from those that would be theoretically correct.

Effect of mass on "metabolic clearance rates." "Metabolic clearance rates" were measured over a 2 hr period in three normal subjects infused with estrone and estradiol labeled either with ^{3}H or ^{14}C. 1 wk later, the studies were repeated but the labels were reversed. The "metabolic clearance rates" were the same (Table V) whether ^{3}H- or ^{14}C- labeled estrogens were used, although the mass of estrogen infused was 600–1000 times greater when the steroid-^{14}C was given. Approximately 25 μg of estradiol-^{14}C and 50 μg of estrone-^{14}C were infused for each study.

The effect of larger amounts of estradiol on the "MCR^{E_2}" and the conversion ratio z^{E_1}/z^{E_3} was examined by giving a continuous infusion of estradiol at varying rates (Table VI). The specific activity of each estradiol infusion was checked by gas-liquid chromatography of ether extracts of the solution and agreed well with the estimated specific activity. The "MCR^{E_2}" remained unchanged when 17 and 170 μg of E_2 were infused daily. When the infusion rate was increased to 1440 μg/day, "MCR^{E_2}" increased 50%. Similarly the conversion ratio was unchanged until the highest infusion rate was reached.

Altered hormonal status. Table VII gives values for "MCR^{E_1}" and "MCR^{E_2}" in six postmenopausal women, three women with elevated cortisol production rates, and a man before and after treatment with fluoxymesterone. If the postmenopausal women are considered as a group, neither "MCR^{E_2}" (634 ±42 liters/24 hr per m^2) nor "MCR^{E_1}" (929 ±72 liters/24 hr per m^2) is different from values obtained in normal women. However, if they are grouped according to number of years after cessation of menses, "MCR^{E_2}" in the late menopausal women (greater than 3 yr) was 553 ±19 liters/24 hr per m^2 whereas early in menopause (less than 1 yr). "MCR^{E_2}" was 732 ±18 liters/24 hr per m^2 ($P < 0.01$). "MCR^{E_1}" was not studied in two of three late menopausal women and therefore no comparison can be made.

The effect of excess glucocorticoids is clearly shown in three patients (Table VII). The corrected "MCR^{E_2}" was 1054 liters/24 hr per m^2. significantly higher than the "MCR^{E_2}" of either normal or postmenopausal women. In the single study with high doses of a synthetic androgen, fluoxymesterone, the "MCR^{E_2}" was increased during therapy.

DISCUSSION

Pearlman (10) introduced the concept that the plasma level of a steroid was related to its production rate by its rate of metabolism. This relationship is now designated as the metabolic clearance rate (1) and it has proved to be a powerful tool in the study of steroid physiology. When the MCR is measured by the constant infusion technique, the length of the infusion must be sufficient to attain steady-state isotope levels in the infused steroid. This requirement has been met in studies with the androgens (4, 5), progesterone (11) and aldosterone (12). Longcope et al. reported that this requirement was

likewise met during 2-hr infusions of estrone and estradiol. We found, however, that tritium levels in plasma estrone did not always become constant during 2-hr estrone-^{3}H infusions and subsequently, 12-hr infusions confirmed that steady-state levels were not attained for at least 9 hr, and that plasma estrone levels at the end of long infusions could be significantly greater than those of the short infusions. A priming dose of the magnitude given before 2-hr studies would not have appreciably changed the tritium levels observed after two hours because of the rapid plasma half-time of estrone. The apparent conflict between these data and those of Longcope et al. (7) may be due to the shorter sampling interval and to the lesser amounts of isotope infused with correspondingly greater counting errors in their study.

It is possible to attach significance to an "MCR" calculated from data obtained during the first 2-hr of estrone-^{3}H infusions? An unequivocal answer to this question is contingent upon elucidation of the reasons for the observed data. At least two possible explanations are consistent with the data: (*a*) that nonsteady-state metabolic processes play a major role in estrone clearance, or (*b*) that the reentry of estrone into the plasma is slow in comparison to its relatively rapid half-time. Steroid-dependent diurnal variation has been demonstrated for the hepatic transaminase system. Similar dependence of the steroid metabolizing enzyme systems could result in failure to reach a steady-state level during long constant infusions. Sandberg and Slaunwhite (13) reported that as much as 2% of the total radioactivity could be found in the blood as estrogen conjugate 48 hr after an injection of estrone-^{3}H. In addition, as much as 50% of the circulating estrogen potentially enters the enterohepatic circulation. Reentry of estrone into the plasma from either or both of these sources could also produce continually increasing plasma tritium levels in estrone. These alternative explanations are not exclusive, though the implications of each are quite different. Failure to have infused long enough to attain steady-state levels means that data from 2-hr infusions lead to an overestimate of the true MCR. On the other hand, lack of conformity to steady-state kinetics would raise serious doubts concerning the validity of a blood production rate calculated from constant infusion data. The wide discrepancy between estrone blood production rates (14) and those calculated from specific activity of

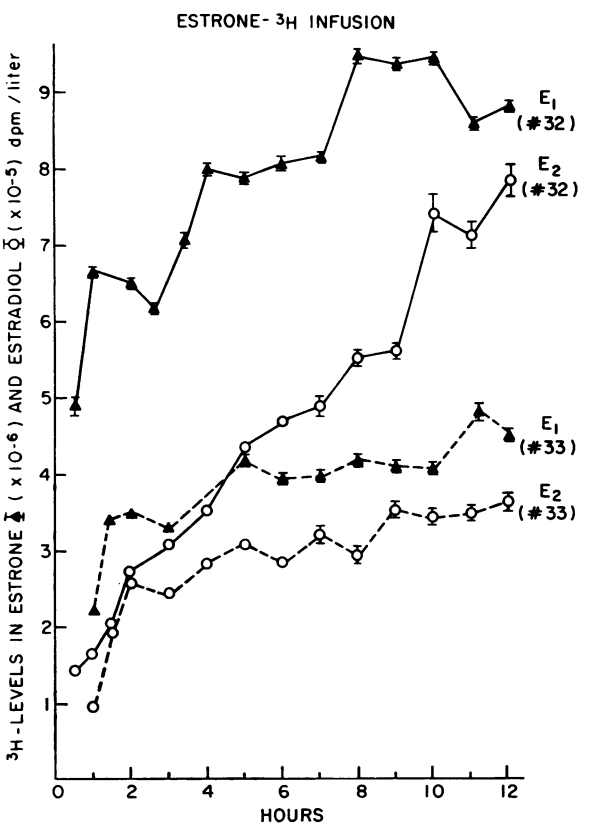

FIGURE 3 Plasma tritium levels in estrone and estradiol during infusion of estrone-^{3}H. Patient Nos. 32 and 33. Brackets enclose 95% confidence limits.

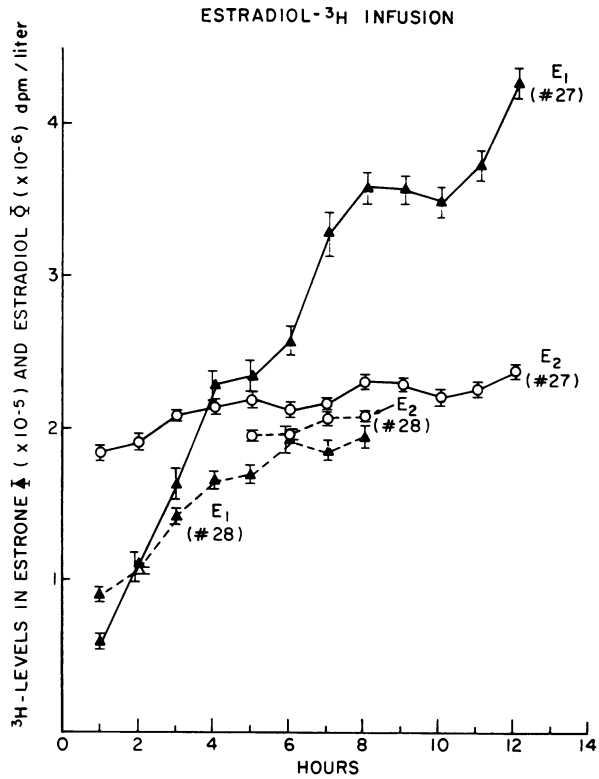

FIGURE 4 Plasma tritium levels in estrone and estradiol during infusion of estradiol-^{3}H. Patient Nos. 27 and 28. Brackets enclose 95% confidence limits.

TABLE V

*Comparison of Estrogen-^{3}H and -^{14}C for the Measurement of "MCR^{E_1}" and "MCR^{E_2}"**

Subject No.	"MCR^{E_1}"		"MCR^{E_2}"	
	^{3}H	^{14}C	^{3}H	^{14}C
	liters/24 hr		*liters/24 hr*	
35	1669	1700	997	1155
36	1625	1368	872	878
37	1823	2096	1143	1106
Mean ±SEM	1706 ±60	1721 ±211	1004 ±78	1046 ±85

* In these studies the plasma isotope levels of infused steroid showed the same deviation from equilibrium as in normal subjects (Tables II and III).

TABLE VI

Effect of Increasing Estradiol Levels on "MCR^{E_2}" and z^{E_1}/z^{E_2} in Patient No. 34*

Day of study	Estradiol infused	Calculated plasma estradiol‡ during infusion	"MCR^{E_2}"	$\frac{z^{E_1}}{z^{E_2}}$§
	μg/day	*ng/100 ml*		
Day 1	Tracer only		1335	0.11 ±0.03
Day 2	17.0	1.2	1465	0.17 ±0.03
Day 4	170	12.8	1323	0.14 ±0.02
Day 6	1444	72.0	1995	0.23 ±0.02
Day 7	Tracer only		1485	0.24 ±0.02

* In these studies the plasma isotope levels of infused steroid showed the same deviation from equilibrium as in normal subjects (Tables II and III).

‡ Calculated from the mean z^{E_2} and the specific activity infused.

§ Conversion fraction, $\frac{z^{E_1}}{z^{E_2}}$ ±95% confidence limits (Appendix II).

urinary metabolites (15, 16) may, in part, be explained by these methodologic considerations.

In contrast to estrone, the tritium levels in estradiol usually became constant 70–120 min after the start of the infusion of estradiol-^{3}H. However, with prolonged infusions, there was a slow rate of increase of plasma tritium levels so that the final plasma levels were significantly greater than the initial ones. Thus, the constant infusion technique probaly gives close to correct values for estradiol "MCR." The differences in the behavior between estrone and estradiol may be attributed to the suggested estrone conjugate pool. The effect of reentry of estrone would be reflected to a much smaller extent in changes in plasma estradiol than in estrone because of the low rate of conversion of estrone to estradiol.

The measurement of the transfer factor [ρ], the fraction of the production rate of a precursor steroid hormone that is converted to a blood or urinary metabolite, is necessary for the analysis of the origins of the steroid

TABLE VII

*Effects of Altered Hormonal Status on MCR^{E_1} and MCR^{E_2}**

Subject No.	Diagnosis	MCR^{E_2}	MCR^{E_2}/m^2	MCR^{E_1}	MCR^{E_1}/m^2
		liters/24 hr	*liters/24 hr per m^2*	*liters/24 hr*	*liters/24 hr per m^2*
23	Early menopause (< 1 yr)	1270	747	1654	973
24		1360	756	1887	1097
25		1228	694	1580	892
	Mean ±SEM		732 ±18		987 ±60
26	Late menopause (> 3 yrs)	816	523	1176	754
27		778	537		
28		815	599		
	Mean ±SEM		553 ±19		
All menopausal women	Mean ±SEM		643 ±42		929 ±72
25	Glucocorticoid excess	2222	1176	2062	1091
29		1560	1006	1520	981
30		1733	979	2275	1285
	Mean ±SEM		1054 ±48		1119 ±73
31	Before fluoxymesterone	1755	848		
	After fluoxymesterone	2614	1269		

* In these studies the plasma isotope levels of infused steroid showed the same deviation from equilibrium as in normal subjects (Tables II and III).

hormones. The use of this concept is best illustrated by the studies of androgen metabolism (4) in which it was possible to measure the amount of blood testosterone derived from precursor androstenedione. In this and comparable studies (5), the plasma levels of isotope in the infused precursor steroid and in its metabolite became constant during the determination of the MCR. When we attempted to measure similarly the transfer factors, $[\rho]_{BB}^{E_1E_2}$ and $[\rho]_{BB}^{E_2E_1}$, plasma tritium levels in the metabolite of the infused steroid did not always become constant during 2-hr infusions. During prolonged infusions, the 50–100% increase in tritium levels in the metabolite and in the conversion fractions indicates that the estimation of these transfer factors from short infusions may be in error to a similar extent. Estrogen transfer factors calculated from the $^3H/^{14}C$ ratios of urinary (U) estrone and estradiol conjugates (17, 18) have been estimated as $[\rho]_{BU}^{E_1E_2} = 0.3–0.5$ and $[\rho]_{BU}^{E_2E_1} = 0.8–1.0$. The previous finding of lower transfer factors for interconversion of blood estrogens (7) was interpreted as suggesting that some of the urinary estrone and estradiol conjugates were derived from other than plasma estrogens. This interpretation may not be warranted in view of the inability to measure blood estrogen transfer factors satisfactorily.

A number of methodologic difficulties have been raised by these studies which may be resolved by elucidation of the role of estrogen conjugates, the enterohepatic circulation and nonsteady-state parameters in the metabolism of estrogens. However, the agreement about the "estrogen MCR's" between two laboratories and the narrow range of values strongly suggest that this parameter measured during 2-hr infusions is reproducible in normal subjects and may be useful in the further study of estrogen metabolism. Therefore, we have made the subsequent comparisons of "estrogen clearance" based on data obtained from the conventional 2-hr infusions.

We have confirmed the previous report that "MCR^{E_2}" is significantly lower than the apparent MCR^{E_1} (P 0.01). In addition, the "MCR^{E_2}" is lower in women than in men whereas there is no sex difference in the apparent "MCR^{E_1}." There is a strong similarity in the metabolism of these interconverting steroids with 17β-hydroxy and 17-ketone functions to the metabolism of the analogous androgen pair, testosterone and androstenedione. Both testosterone (19) and estradiol (20) are bound to a specific plasma protein and the metabolic clearance rate of both is lower than that of androstenedione and estrone which are less strongly bound to plasma proteins. Further, with both estradiol and testosterone, the metabolic clearance rate is lower in women than in men (5). Protein binding of testosterone is greater in women than in men and a similar difference has been suggested by the sex difference in the distribution of estradiol between red cells and plasma (7). It is tempting to relate these differences in the clearance rate to differences in the degree of protein binding. However metabolic clearance rates are the composite of multiple chemical reaction rates and rates of transfer among compartments. Sex differences in the pattern of the urinary excretion of metabolites of estradiol have been noted (21, 22). In androgen studies, Southren, Gordon, and Tochimoto (23) suggested that testosterone induced increases of metabolic clearance rates must be due, in part, to increased hepatic metabolism rather than changes only in plasma binding of testosterone.

If the sex differences observed in "MCR^{E_2}" are related to plasma estradiol levels, then the "MCR^{E_2}" might be higher in postmenopausal women than in normal menstruating women. Since the mean "MCR^{E_2}" of the six postmenopausal women did not differ from that of the younger women, no support was obtained for this hypothesis. The limited sample studied precludes any conjectures about such possibly important factors as length of time after the menopause and biologic age.

The minimal effects of acute changes in plasma estrogen concentrations on the "MCR^{E_2}" were apparent from those studies in which "MCR^{E_2}" was determined during infusions of varying amounts of estrogen. The data show that E_2-^{14}C can serve as an adequate tracer for "MCR" determination although the estradiol infusion rate was equivalent to 300 μg/24 hr. Similarly, the infusion of estradiol to produce estradiol levels of 7 ng/100 ml and 17 ng/100 ml comparable to those seen in normal men and women did not alter estradiol metabolic clearance rates. When the calculated plasma estradiol reached a concentration of 72 ng/100 ml, a level compatible with that of preganancy, "MCR^{E_2}" increased sharply and returned to normal 48 hr after cessation of the estrogen infusion. In contrast with these acute studies it might be anticipated that prolonged treatment with estrogens would decrease the "metabolic clearance" of estradiol since the metabolic clearance rates of both testosterone (23) and cortisol are decreased by estrogen treatment. Although prolonged estrogen treatment increases the binding capacity of estradiol-binding globulin an independent effect of estrogen on tissue extraction has not been eliminated.

The interpretation of the increased "MCR^{E_2}" during fluoxymesterone therapy is tenuous at present but an increased clearance could result from any one of the following effects of androgen treatment: (*a*) displacement of estradiol from plasma protein by fluoxymesterone, (*b*) increased tissue extraction due to enzyme induction, or (*c*) suppression of the estradiol-binding protein. It is of interest to note that fluoxymesterone treatment as well as increased endogenous testosterone production

will increase the testosterone metabolic clearance rate in women.

Dexamethasone treatment of hirsute women produces a small but definite increase in testosterone metabolic clearance rates (24). In the present study we have demonstrated a similar effect of glucocorticoid excess on "MCR^{E_2}". The mechanism by which the clearance of testosterone and estradiol are altered by glucocorticoids is not understood. However, the study of the adrenalectomized woman indicates that this effect is a result of increased corticoid production rather than increased ACTH or adrenal androgen production.

APPENDIXES

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I. *Statistical analyses of the $^3H/^{14}C$ ratios.* The formula used to determine the $^3H/^{14}C$ ratio, R, corrected for background is

$$R = \frac{X_H - X_{BH} - fX_C}{X_C - X_{BC}},$$

where X_H is the per minute count on tritium; X_{BH} is the per minute count on the tritium background; X_C is the per minute count on carbon-14; X_{BC} is the per minute count on the carbon-14 background; and, f is the proportion of runover of the carbon-14 into the tritium window.

Each per minute count is based on counts of N minutes, where N is large eough so that each of the total counts (NX_H, NX_{BH}, etc.) is large, say at least 100. We also assume that all the X's are independent Poisson variates so that the variances of the X's may be estimated by X/N. Furthermore f is assumed to be a constant free from statistical variation for any particular calculation of R.

Since R is a ratio, it is somewhat simpler to base its statistical analysis on its natural logarithm. We easily find, using the usual Taylor series expansion argument, that its approximate variance is given by,

$$\mathrm{Var}(\ln R) = \frac{1}{N}\left\{\frac{X_H + X_{BH} + f^2X_C}{(X_H - X_{BH} - fX_C)^2} + \frac{X_C + X_{BC}}{(X_C - X_{BC})^2} + \frac{2fX_C}{(X_H - X_{BH} - fX_C)(X_C - X_{BC})}\right\},$$

and its standard error is $\mathrm{SE}(\ln R) = \sqrt{\mathrm{Var}(\ln R)}$. This number can be used to calculate a 95% confidence interval for R, with lower limit, $R\exp[-1.96\mathrm{SE}(\ln R)]$, and upper limit, $R\exp[+1.96\mathrm{SE}(\ln R)]$.

If we wish to find 95% confidence limits for the ratio of two such independent ratios, R and R', the lower limit is $(R/R')\exp[-1.96\sqrt{\mathrm{Var}(\ln R)+\mathrm{Var}(\ln R')}]$ and the upper limit is $(R/R')\exp[+1.96\sqrt{\mathrm{Var}(\ln R)+\mathrm{Var}(\ln R')}]$.

A simple chi-square test for the comparison of two independent estimates, R and R', can be based on the statistic,

$$\chi^2 = \frac{(\ln R - \ln R')^2}{\mathrm{Var}(\ln R) + \mathrm{Var}(\ln R')},$$

which is significant at the 5% level if χ^2 exceeds 3.84.

If the same background counts are used to adjust both R and R', the statistical test must account for this fact. The adjusted statistic is

$$\chi^2 = \frac{(\ln R - \ln R')^2}{\mathrm{Var}(\ln R) + \mathrm{Var}(\ln R') - 2\,\mathrm{Cov}(\ln R, \ln R')}$$

where

$$\mathrm{Cov}(\ln R, \ln R') = \frac{1}{N}\left(\frac{X_{BH}}{(X'_H - X_{BH} - fX'_C)(X_H - X_{BH} - fX_C)} + \frac{X_{BC}}{(X_C - X_{BC})(X'_C - X_{BC})}\right).$$

The covariance term will generally be comparatively small.

We illustrate the above statistical methods with numerical examples. If $X_H = 20$, $X_{BH} = 10$, $X_C = 30$, $X_{BC} = 7$, $f = 0.18$, and $N = 100$, then

$$R = \frac{20 - 10 - (0.18)(30)}{30 - 7} = 0.200,$$

and

$$\mathrm{Var}(\ln R) = \frac{1}{100}\left[\frac{20 + 10 + (0.18)^2(30)}{[20 + 10 - (0.18)(30)]^2} + \frac{30 + 7}{(30 - 7)^2} + \frac{2(0.18)(30)}{[20 - 10 - (0.18)(30)](30 - 7)}\right] = 0.002232.$$

$$\mathrm{SE}(\ln R) = \sqrt{0.002232} = 0.047.$$

Lower 95% limit $= (0.200)\exp[(-1.96)(0.047)] = 0.182.$

Upper 95% limit $= (0.200)\exp[(+1.96)(0.047)] = 0.219.$

If we are comparing two independent ratios, say, R = 0.200 with Var(lnR) = 0.002232 and R' = 0.250 with Var(lnR) = 0.002668, the chi-square statistic for possible difference is

$$\chi^2 = \frac{[\ln(0.250) - \ln(0.200)]^2}{0.002232 + 0.002668} = 10.16,$$

which is highly significant.

II. *Statistical analyses of conversion fraction $C^{E_1E_2}$ and transfer factor $\rho^{E_1E_2}$.* The statistical analysis of the conversion fraction, $C^{E_1E_2} = X^{E_2}/X^{E_1}$, is also most easily done by considering its logarithm. Proceeding as in Appendix I, we find

$$\mathrm{SE}(\ln C^{E_1E_2}) = \sqrt{\frac{\mathrm{Var}(X^{E_2})}{(X^{E_2})^2} + \frac{\mathrm{Var}(X^{E_1})}{(X^{E_1})^2}},$$

where the $\mathrm{Var}(X^{E_1})$ and $\mathrm{Var}(X^{E_2})$ are computed in the usual way from the multiple determinations of X^{E_1} and X^{E_2} at equilibrium. The lower 95% confidence limit is $C^{E_1E_2}\exp[-1.96\mathrm{SE}(\ln C^{E_1E_2})]$ and the upper 95% limit is $C^{E_1E_2}\exp[+1.96\mathrm{SE}(\ln C^{E_1E_2})]$.

The transfer factor, $\rho^{E_1E_2} = C^{E_1E_2}\,\mathrm{MCR}^{E_2}/\mathrm{MCR}^{E_1}$, is statistically analyzed by considering its natural logarithm. We find,

$$\mathrm{SE}(\ln\rho^{E_1E_2}) = \sqrt{\frac{\mathrm{Var}(C^{E_1E_2})}{(C^{E_1E_2})^2} + \frac{\mathrm{Var}(\mathrm{MCR}^{E_2})}{(\mathrm{MCR}^{E_2})^2} + \frac{\mathrm{Var}(\mathrm{MCR}^{E_1})}{(\mathrm{MCR}^{E_1})^2}}$$

and the confidence limits are found analogous to that of $C^{E_1E_2}$.

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