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Research Article

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Metabolic Clearance Rates and Interconversions of Estrone and 17β-Estradiol in Normal Males and Females

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ABSTRACT The continuous infusion of ^{3}H-6,7-estrone and ^{3}H-6,7-estradiol has been used to study the metabolic clearance rate (MCR), the interconversions, and the red cell uptake of these steroids in normal males and females. The whole blood MCR of estrone is 1,990 ± 120 liters per day/m^2 (SE) in males and 1,910 ± 100 liters per day/m^2 in females. The whole blood MCR of estradiol is 1,600 ± 80 liters per day/m^2 in males and 1,360 ± 40 liters per day/m^2 in females. The values in females do not vary significantly when studied in the follicular or luteal phase of the cycle. At least 35% of the total estrone metabolism in both sexes is extrasplanchnic and at least 25% of the total estradiol metabolism in males, and 15% in females is extrasplanchnic. The $[\rho]_{BB}^{2,1}$ [transfer constant of estradiol to estrone, which is equivalent to the fraction of the precursor (estradiol) converted to the product (estrone) when both the infusion of the precursor and the measurement of the product are in peripheral blood] is 15%; and the $[\rho]_{BB}^{1,2}$ [transfer constant of estrone to estradiol, which is equivalent to the fraction of the precursor (estrone) converted to product (estradiol) when both the infusion of the precusor and the measurement of the product are in peripheral blood] is 5% in both males and females. Our findings concerning the radioactivity in whole blood, as measured by our procedure, were the following: 15–20% of estrone in both sexes and 15% of estradiol in males is associated with red cells. Only 2% of the whole blood radioactivity of estradiol in females is associated with red cells. Changes in the distribution of radioactivity between plasma and red cells will influence the MCR as calculated from plasma, but not as calculated from whole blood.

INTRODUCTION

The metabolic clearance rate (MCR) of a substance, defined as that volume of blood from which it is totally and irreversibly cleared in unit time, is a concept which has been applied to studies of the metabolism of aldosterone, cortisol, progesterone, and the androgens (1–5). In this manner, information has been obtained concerning splanchnic and extrasplanchnic metabolism. In addition, the use of the continuous infusion technique has provided data on the interconversion of androstenedione and testosterone (3, 4, 6) and on the uptake of steroid hormones into red cells (2).

Previous studies of the metabolism of estrone and 17β-estradiol in nonpregnant humans have provided some data concerning the half-lives of these steroids in plasma (7) and the red cell binding of estrone (8). The major studies have been concerned with urinary metabolites and with the radioactivity appearing in these metabolites after administration of the labeled hormone (9–11). In-

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formation obtained in this manner however does not always reflect the dynamics of the circulating hormone (4, 12, 13). Therefore, we felt that a study of the metabolic clearance rates and the interconversion of estrone and 17β-estradiol in blood would provide a fuller picture of the metabolism of these steriods.

The plasma MCR of testosterone is much lower than the hepatic plasma flow. This difference indicates incomplete splanchnic extraction for this steroid (3, 4, 6). However, the plasma MCR of androstenedione is greater than hepatic plasma flow (3, 4, 6). Bardin and Lipsett (13) have reported that the plasma MCR of testosterone is greater for males than for females, and the difference is significant after correction for body surface areas. There is, however, no sex difference in the MCR per square meters of body surface area of androstenedione. These data could be explained by binding of testosterone to plasma proteins other than albumin, provided that such binding was greater in plasma from females than from males, and relatively greater than the protein binding of androstenedione in plasma from both sexes. We were, therefore, particularly interested in the possibility of an analogous situation existing for estradiol and estrone.

METHODS

Normal adults aged 21–40 yr who were informed and gave their consent were used for all studies. All subjects were in good health, and were receiving no medications; the females all gave a history of normal menstrual cycles.

Methylene chloride, acetone, chloroform, and benzene were of spectral quality. All other solvents were of technical grade and were redistilled prior to use. Pyridine was prepared as described (14). Acetic anhydride was distilled, and the fraction boiling at 138°C was collected.

^{3}H-6,7-estrone and ^{3}H-6,7-17β-estradiol (34 c/mmole) were obtained from the New England Nuclear Corp., and 4-^{14}C-estrone (33.7 mc/mmole) and 4-^{14}C-17β-estradiol (31.7 mc/mmole) were obtained from the Nuclear-Chicago Corporation. They were separately purified prior to use by partition column chromatography on Celite using the E-1 and E-2 systems of Siiteri (15). The tubes containing the radioactive peak from each column were pooled and the contents were chromatographed on Whatman No. 2 paper in a Bush-type system (stationary phase: methanol, water, 3:1; mobile phase: toluene). The radioactive peak was located by a Vanguard 880 strip scanner, cut out, and eluted with acetone. The steroids were stored at −15°C in MeOH-benzene, 1:9, v/v. Tritium-labeled steroids were purified every 3–4 wk, the ^{14}C-labeled steroids every 4 months. The purity of the radioactive steroids was checked as follows: ^{3}H-estrone and 4-^{14}C-estrone were mixed, and an aliquot was removed for counting; another aliquot was chromatographed on Whatman No. 2 paper in the Bush-type system (toluene, methanol, water, 4:3:1), and a third aliquot was acetylated with pyridine and acetic anhydride, and the resultant estrone acetate was chromatographed on thin-layer chromatography (TLC) in two dimensions in system II (see below). The ^{3}H/^{14}C ratios of the three samples were 27.52, 27.61, and 27.69, respectively. Similarly, the ^{3}H-estradiol and the ^{14}C-estradiol were mixed and an aliquot counted; another aliquot was acetylated with pyridine and acetic anhydride and the resultant estradiol-3-monoacetate chromatographed on TLC in two dimensions in systems III and IV (see below). The ^{3}H/^{14}C ratios obtained were 53.26 and 53.11. These ratios indicate the purity of infused steroids.

Experiments were begun between 6 and 8 a.m. with the subjects in a fasting state and supine for 12 hr before and throughout the experiment. A priming dose of 4 μc of one of the ^{3}H-labeled steroids in 10 ml of 8% ethanol in an isotonic saline solution was injected into an arm vein at 0 min. The continuous infusion was begun 30 min later with 4 μc of the same ^{3}H-steroid in 25 ml of 8% ethanol in an isotonic saline solution infused into an arm vein at 0.190 ml/min by a 50 ml Yale glass syringe in a Harvard infusion pump.[1] The tubing used was Teflon,[2] and adsorption was negligible. Samples were taken from the priming dose and from the end of the infusion tubing both before and after the infusion. Aliquots of these samples were counted as described by Flood and his coworkers (16), and internal standards were used to correct for quenching.

Estradiol was infused for 105 min, and blood samples were drawn at 105, 120, and 135 min from time of priming dose. Estrone was infused for 120 min, and blood samples were drawn at 120, 135, and 150 min from time of priming dose. When it was established that equilibrium had been reached at these times (see below), only two blood samples were drawn: 20 min from the end of the infusion and at its end.

All blood samples were drawn into 50-ml heparinized, disposable syringes from a vein in the arm opposite from the infusion. The blood was transferred to chilled tubes and centrifuged, usually at 4°C, for 30 min. 10 ml of plasma was analyzed as described below. When whole blood clearance rates were determined, an aliquot of whole blood was removed initially from the tube for estimation of the hematocrit, and 10 ml of whole blood was removed for analysis as described below.

Thin-layer chromatography. All TLC was carried out on 20 × 20 cm glass plates using HF_{254} Silica Gel [3] 0.250 mm thick. Carrier estrone [4] and estradiol [4] were located

[1] Harvard Apparatus Co., Inc., Dover, Mass.

[2] Becton-Dickinson & Co., Rutherford, N. J.

[3] Distributed by Brinkmann Instruments Inc., Westbury, L. I.

[4] Obtained from Steraloids, Inc., Pawling, N. Y.

by absorption at 277 mμ using a mercury lamp and a combination liquid cell (17). Carrier estrogen acetates [4] were located by absorption at 254 mμ using a standard 254 mμ source. Elution of the steroids from TLC was carried out in the same manner as described by Horton and Tait (3), except that we used 6 ml of acetone. The solvent systems used for TLC were as follows:

I. chloroform : acetone	85 : 15
II. chloroform : acetone	92 : 8
III. chloroform : acetone	97 : 3
IV. benzene : ethanol	98 : 2
V. cyclohexane : ethyl acetate	75 : 25

Extraction from plasma. 10 ml of plasma was added to flasks containing 4-^{14}C-estrone (120 dpm) and 4-^{14}C-estradiol (120 dpm) in 0.2 ml EtOH. 1 ml of 1.25 N NaOH and 2.5 ml of saturated $NaHCO_3$ were added to these flasks. The contents were swirled and then added to a 500 ml separatory funnel and extracted twice with 60 ml volumes of ice-cold methylene chloride (CH_2Cl_2). The CH_2Cl_2 extracts were pooled and washed twice with 20 ml of distilled water. The CH_2Cl_2 was removed under vacuum, and the residue was transferred to 10-ml glass-stoppered tubes and partitioned between 3.0 ml 1 N NaOH and 0.5 ml benzene. The benzene was discarded, and the alkaline extract was transferred to 60-ml separatory funnels, and 6.0 ml of saturated $NaHCO_3$ was added. This solution was extracted twice with 10-ml portions of CH_2Cl_2 which were pooled and washed once with 2.0 ml H_2O and three times with 1.0 ml volumes of H_2O. The CH_2Cl_2 was removed under a stream of nitrogen, and the residue was spotted on a thin-layer plate with 20 μg of nonradioactive carrier estrone and 17β-estradiol. System I was used for this chromatography, after which the steroids were eluted and concentrated in the bottom of 10-ml glass-stoppered tubes.

To each tube pyridine 0.2 ml and 0.005 ml of 10% acetic anhydride in benzene were added. The tubes were stoppered and placed overnight at room temperature in a desiccator. Under these conditions estrone was converted to estrone acetate, and estradiol was converted to estradiol-3-monoacetate in 80–90% yield (18). The reactions were stopped with 0.05 ml of ethanol, and the tubes dried under a stream of nitrogen. The acetates were separately analyzed in two dimensions by TLC: estrone acetate in system III and estradiol-3-monoacetate in system II. 20 μg of the appropriate unlabeled estrogen acetate was added to each plate as carrier. The steroids were located and eluted into counting vials. The vials were dried in a vacuum oven, and 0.2 ml of methanol and 10 ml of scintillation fluid (84 ml of Liquifluor,[5] 20 ml of ethanol, 1 liter of toluene) were added to each vial. The vials were counted for 5 × 50 min. Using a treatment similar to that described by Horton and Tait (19), we can calculate, for minimal total counting times of 250 min, that the maximal errors for the determination of the concentrations of radioactivity as the precursor and the product steroid would be 2 and 15%, respectively.

Extraction of whole blood. 10 ml of whole blood was added to a flask containing 4-^{14}C-estrone (120 dpm) and 4-^{14}C-estradiol (120 dpm) in 0.2 ml of ethanol. The contents of the flask were transferred to a 500 ml separatory funnel and the flask rinsed successively with 10 ml distilled water and 10 ml diethyl ether. The rinses were added to the funnel, and the contents were extracted twice with 100-ml volumes of ice-cold CH_2Cl_2. The CH_2Cl_2 extracts were pooled and washed three times with 30-ml volumes of water. The CH_2Cl_2 was removed under vacuum, and the same steps followed as were previously described under Plasma.

Recoveries. Over-all recoveries of 4-^{14}C-estrone added to plasma or whole blood were 45–55%, and recoveries of 4-^{14}C-estradiol were 50–60%.

Conversion in the method. To check the possibility of conversion of the estrogen occurring after the blood was drawn, ^{3}H-estradiol was added to whole blood and processed as described above. In these experiments radioactivity was recovered as estradiol, but the area corre-

[5] Liquifluor obtained from Pilot Chemicals, Inc., Watertown, Mass.

TABLE I

$^3H/^{14}C$ Ratios of Derivatives Formed in Tests of Radiochemical Purity

	Estradiol 3-monoacetate*	Estradiol diacetate*	Estrone acetate*	Estradiol 3-monoacetate*
Estradiol infusion				
Estradiol as	3.60 ± 0.05	3.56 ± 0.5		
	2.45 ± 0.05	2.49 ± 0.05		
Estrone as			0.29 ± 0.01	0.34 ± 0.02
			0.17 ± 0.02	0.17 ± 0.01
Estrone infusion				
Estradiol as	0.221 ± 0.004	0.290 ± 0.006		
	0.307 ± 0.010	0.300 ± 0.010		
Estrone as			2.69 ± 0.05	2.99 ± 0.06
			3.88 ± 0.06	4.17 ± 0.06

* Values listed are $^3H/^{14}C$ ratios ± statistical counting error.

TABLE II

Data for the Determination of Plasma MCR^1, MCR^2, x^2/x^1, z^1/z^2, $[\rho]_{BB}$ in males

Subjects	cpm/day infused ^{3}H-estrone	cpm/liter plasma* estrone (x^1) after priming dose: 120 min	135 min	150 min	Mean	MCR^1‡	cpm/liter plasma estradiol (x^2) after priming dose: 120 min	135 min	150 min	Mean	x^2/x^1	$[\rho]_{BB^{1,2}}$§
						liters/day						
1. J.M.	37.3×10^6	13,670	14,750	15,690	14,700	2,540	960	760	980	900	0.061	0.045
2. A.G.	26.4×10^6	10,430	10,550	11,970	10,980	2,400	590	510	740	610	0.055	0.043
3. K.J.H.	26.6×10^6	14,610	17,070	15,150	15,600	1,700	785			785	0.050	0.055
4. P.D.	25.4×10^6	7,380		8,510	7,950	3,200	680		710	690	0.087	0.051
5. A.C.	24.9×10^6	9,410		9,650	9,530	2,620	530		700	610	0.064	0.047
6. G.R.	10.2×10^6		4,110	4,270	4,270	2,430		320	420	370	0.088	0.069
7. D.O.	18.8×10^6		8,640	8,260	8,450	2,220		320	470	390	0.046	0.039
8. A.D.	16.2×10^6		5,530	4,840	5,180	3,130		480	520	500	0.096	0.059
9. D.C.	26.3×10^6		8,740	9,330	9,040	2,910		640	680	660	0.073	0.047
Mean±SE						2,570±160					0.069±0.002	0.050±0.004

Subjects	cpm/day infused ^{3}H-estradiol	cpm/liter plasma* estradiol (z^2) after priming dose: 105 min	120 min	135 min	Mean	MCR^2‡	cpm/liter plasma estrone (z^1) after priming dose: 105 min	120 min	135 min	Mean	z^1/z^2	$[\rho]_{BB^{2,1}}$‖
1. J.M.	22.9×10^6	10,810	12,690	13,090	12,200	1,870	1,290	1,390	1,350	1,340	0.11	0.151
2. I.N.	24.4×10^6	13,740	13,760	12,920	13,470	1,810	2,150	2,090	2,170	2,130	0.16	0.224
3. D.P.	30.5×10^6	17,790	19,280	20,100	19,100	1,600	1,170	1,110	1,520	1,270	0.063	0.101
4. K.J.H.	35.7×10^6	25,140	27,700	27,490	26,800	1,330	2,920	2,880	2,680	2,830	0.11	0.204
5. B.G.	30.5×10^6	17,790	17,060	18,780	17,880	1,710	1,230		1,690	1,460	0.082	0.123
6. E.N.	22.8×10^6	12,450		12,000	12,220	1,870	1,700		1,300	1,500	0.12	0.168
7. C.L.	17.8×10^6		8,050	8,670	8,360	2,130		1,010	980	990	0.12	0.143
8. A.C.	24.8×10^6		12,360	12,970	12,600	1,960		860	1,070	960	0.076	0.099
9. D.C.	24.2×10^6		11,060	12,150	11,600	2,090		1,550	1,820	1,680	0.14	0.178
10. F.B.	15.3×10^6		8,300	8,560	8,430	1,810		1,050	890	970	0.11	0.163
11. L.S.	17.9×10^6		6,830	6,940	6,880	2,610		1,120	1,110	1,110	0.16	0.158
Mean±SE						1,890±100					0.11 ±0.01	0.156±0.012

* ^{3}H (counts per minute per liter) corrected for losses with ^{14}C indicator.

* ^{3}H counts per sample before correction for recoveries were 0.5% of these figures.

‡ MCR^1 and MCR^2 metabolic clearance rate of estrone and estradiol.

§ $[\rho]_{BB^{1,2}}$ calculated as $(MCR^2/MCR^1)\,x^2/x^1$ using the individual's own MCR^1 and x^2/x^1 and the mean male value for MCR^2 of 1890 liters/day.

‖ $[\rho]_{BB^{2,1}}$ calculated as $(MCR^1/MCR^2)z^1/z^2$ using the individual's own MCR^2 and z^1/z^2 and the mean male value for MCR^1 of 2570 liters/day.

sponding to the carrier estrone had background radioactivity only. The reverse experiment yielded similar results showing that no interconversion of these estrogens occurred under the conditions we used.

Radiochemical purity of recovered estrone and estradiol. In four experiments, two involving estrone infusions and two involving estradiol infusions, the plasma extracts representing the last two time intervals were pooled and, following the formation and two-dimensional TLC of the acetates of estrone and estradiol, aliquots were removed for counting and for further derivative formation.

Estrone acetate was converted to estradiol-3-monoacetate, with 125 μg $NaBH_4$ in 80% dioxane (20). The reaction was carried out for 30 min at 0°C and stopped with 0.05 ml of glacial acetic acid. The residues were dried under a stream of nitrogen and chromatographed on Whatman No. 1 paper in a Bush-type system (stationary phase: methanol, water, 9:1; mobile phase: hexane). Standards were run along with the samples and sprayed with 10% phosphomolybdic acid in MeOH in order to locate the steroids. The area corresponding to authentic estradiol-3-monoacetate was recovered and eluted into a counting vial and counted as described below.

An aliquot of estradiol-3-monoacetate from the two-dimensional TLC was removed for counting, and the remainder was reacted with equal volumes of pyridine and 10% acetic anhydride in benzene to form estradiol diacetate. The diacetate was chromatographed with a nonradioactive carrier in one-dimension in TLC system V and located under 254 mμ ultraviolet light. The compound was eluted into counting vials and counted. The results are shown in Table I. Although in two experiments the ^{3}H/^{14}C ratios increased slightly more than to be expected from statistical counting errors alone, in no case did the ratios fall. If the ^{3}H steroids had been impure, the ^{3}H/^{14}C ratios would have decreased on forming the second derivative. Since the ^{3}H/^{14}C ratios

stayed the same or rose slightly, we feel we achieved radiochemical purity.

Evidence that equilibrium was established for precursor. One of the criteria for the determination of the MCR is that the concentration of radioactivity, corrected for recoveries, of the infused steroid (the precursor) is at equilibrium when the blood samples are drawn. The concentrations of the first and third blood sample com-

TABLE III

Metabolic Clearance Rates Expressed in Plasma and Whole Blood with and without Correction for Body Surface Area

No. of subjects	Sex	Steriod	Plasma MCR	Plasma MCR	Whole blood MCR	Whole blood MCR
			*liters/day**	*liters/day per m²**	*liters/day**	*liters/day per m²**
(9)	Male	Estrone	2,570 ± 160	1,310 ± 80	3,880 ± 220	1,990 ± 120
(10)	Female	Estrone	2,210 ± 120	1,320 ± 70	3,200 ± 170	1,910 ± 100
(11)	Male	Estradiol	1,890 ± 100	990 ± 50	3,060 ± 160	1,600 ± 80
(13)	Female	Estradiol	1,350 ± 40	790 ± 20	2,280 ± 90	1,360 ± 40

* All values ± SE.

TABLE IV

Data for the Determination of Plasma MCR^1, MCR^2, x^2/x^1, z^1/z^2, $[\rho]_{BB}$ in Females

Subjects	Day of cycle	cpm/day infused as ^{3}H-estrone	cpm/liter plasma* estrone (x^1) after priming dose: 120 min	135 min	150 min	Mean	MCR^1‡	cpm/liter plasma estradiol (x^2) after priming dose: 120 min	135 min	150 min	Mean	x^2/x^1	$[\rho]_{BB^{1,2}}$§
1. C.R.	7	14.0×10⁶	6,240	5,430	5,350	5,670	2,470	310	370	320	330	0.058	0.032
2. K.B.	2	30.1×10⁶	15,520	16,280	16,280	16,150	1,860	990	820	1,080	960	0.059	0.043
3. S.P.	12	24.1×10⁶		10,010	9,750	9,880	2,440		310	490	400	0.040	0.022
4. P.B.	8	20.1×10⁶	11,030	10,310	11,290	10,880	1,840	750	690	760	730	0.067	0.049
5. A.B.	6	23.2×10⁶		14,710	14,680	14,690	1,580		1,260	990	1,120	0.076	0.065
6. S.M.	10	21.8×10⁶		10,010	10,660	10,380	2,100		700	720	710	0.068	0.044
7. J.D.	27	24.5×10⁶		11,570	11,070	11,320	2,160		740	740	740	0.065	0.041
8. M.T.	24	29.8×10⁶		11,220	13,630	12,420	2,400		780	570	620	0.050	0.028
9. J.P.	24	17.7×10⁶		6,640	5,650	6,140	2,880		790	580	680	0.111	0.052
10. P.R.	23	17.4×10⁶		7,770	6,960	7,360	2,360		360	400	380	0.052	0.030
Mean ± SE							2,210 ± 120					0.065 ± 0.016	0.041 ± 0.004

Subjects	Day of cycle	cpm/day infused as ^{3}H-estradiol	cpm/liter plasma* estradiol (z^2) after priming dose: 105 min	120 min	135 min	Mean	MCR^2‡	cpm/liter plasma estrone (z^1) after priming dose: 105 min	120 min	135 min	Mean	z^1/z^2	$[\rho]_{BB^{2,1}}$‖
1. A.B.	6	22.4×10⁶	17,520	17,790	17,400	17,570	1,270	2,680	2,450	2,320	2,480	0.141	0.245
2. P.W.	4	19.9×10⁶		12,540		12,540	1,590		980	570	770	0.061	0.085
3. S.B.	7	25.8×10⁶		17,340	17,020	17,180	1,500		1,770	1,700	1,730	0.101	0.148
4. J.D.	4	25.6×10⁶		16,870	17,640	17,250	1,480		1,920	2,580	2,250	0.130	0.194
5. S.M.	4	16.9×10⁶		13,100	13,390	13,390	1,260		1,940	2,470	2,200	0.164	0.287
6. E.Wo.	24	16.8×10⁶	13,930	14,610	14,570	14,370	1,170		1,180	1,170	1,170	0.081	0.153
7. K.B.	24	25.1×10⁶	16,360	15,910	15,840	16,040	1,570	2,110	1,980	2,370	2,150	0.134	0.189
8. E.W.	16	22.7×10⁶	19,260	18,270	19,470	19,000	1,200	1,260	1,140	1,500	1,300	0.068	0.125
9. C.F.	17	17.9×10⁶	12,400	12,640		12,520	1,430	1,600	1,260	1,430	1,430	0.114	0.175
10. M.T.	22	18.9×10⁶	17,720	14,420	15,970	16,040	1,180	1,370	1,690		1,530	0.093	0.174
11. S.P.	6	19.7×10⁶		15,900	13,290	14,590	1,350		1,910	1,960	1,930	0.132	0.216
12. E.M.	21	20.9×10⁶		18,670	19,230	18,950	1,100		1,480	1,530	1,500	0.079	0.159
13. J.P.	3	24.8×10⁶		17,950	17,460	17,700	1,400		1,020	1,200	1,110	0.063	0.100
Mean ± SE							1,350 ± 40					0.105 ± 0.009	0.173 ± 0.017

* ^{3}H (counts per minute per liter) corrected for losses with ^{14}C indicator.

* ^{3}H counts per sample before correction for recoveries were 0.5% of these figures.

‡ MCR^1 and MCR^2 = metabolic clearance rate of estrone and estradiol.

§ $[\rho]_{BB^{1,2}}$ calculated as $(MCR^2/MCR^1)\ x^2/x^1$ using the individuals' own MCR^1 and x^2/x^1 and the mean female MCR^2 value of 1,350 liters/day.

‖ $[\rho]_{BB^{2,1}}$ calculated as $(MCR^1/MCR^2)\ z^1/z^2$ using the individual's own MCR^2 and z^1/z^2 and mean female MCR^1 value of 2,210 liters/day.

TABLE V

Metabolic Clearance Rates, CR, $[\rho]_{BB}$ in Females in Follicular and Luteal Phases of the Cycle, in Females without Regard to Cycle Phase, and in Males

No. of subjects	Sex	Cycle phase	Steriod	MCR^{1}*	x^2/x^1*	$[\rho]_{BB^{1,2}}$*‡
				liters/day		
(7)	F	Follicular	Estrone	2,000 ± 130	0.058 ± 0.005	0.041 ± 0.005
(5)	F	Luteal	Estrone	2,350 ± 150	0.067 ± 0.011	0.036 ± 0.004
(10)	F	Grouped	Estrone	2,210 ± 120	0.065 ± 0.016	0.041 ± 0.004
(9)	M	Grouped	Estrone	2,570 ± 160	0.069 ± 0.002	0.050 ± 0.004
				MCR^{2}* *liters/day*	z^1/z^2*	$[\rho]_{BB^{2,1}}$*
(9)	F	Follicular	Estradiol	1,360 ± 60	0.114 ± 0.012	0.175 ± 0.024
(6)	F	Luteal	Estradiol	1,280 ± 70	0.095 ± 0.010	0.173 ± 0.010
(13)	F	Grouped	Estradiol	1,350 ± 40	0.105 ± 0.009	0.173 ± 0.017
(11)	M	Grouped	Estradiol	1,890 ± 100	0.11 ± 0.01	0.156 ± 0.012

* All values ± SE.

‡ Calculated as per Tables II and III using respective MCR^1 and MCR^2 values for each group.

pared to the second sample after an infusion of estrone were 100 ± 3% (SE) and 102 ± 2%; after an infusion of estradiol, the results were 101 ± 3% and 102 ± 3%. None of the values differed significantly from 100%, and there was no trend in the values.

Evidence that equilibrium was established for product. Where the cpm, corrected for recovery, of the product were similarly evaluated, the concentrations of the first and third sample of estradiol (product) following an infusion of estrone were 104 ± 3% and 106 ± 5%; following an infusion of estradiol, the concentrations of estrone (product) were 102 ± 6% and 109 ± 7%. None of the values differed significantly from 100%, and there was no trend in the values.

RESULTS

All values quoted are mean ± SE unless stated otherwise.

Plasma metabolic clearance rates in males (Table II). The mean plasma MCR for estradiol in 11 males is 1,890 ± 100 liters/day. The mean plasma MCR for estrone in 9 males is 2,570 ± 160 liters/day. When these values are corrected for body surface area (Table III), the MCR's are 990 ± 50 liters/day per m^2 and 1,310 ± 80 liters/day per m^2 respectively. The values for estrone are significantly greater than those for estradiol whether or not they are corrected for body surface area ($P < 0.01$ t test). The MCR for both steroids was determined in four subjects on separate occasions as shown in Table II, and all these four subjects showed a higher MCR as liters of plasma/day or liters of plasma/day per m^2 for estrone than for estradiol.

Plasma MCR's in females (Table IV). The mean plasma MCR for estradiol in 13 females is 1,350 ± 40 liters/day and the mean plasma MCR for estrone in 10 females is 2,210 ± 120 liters/day. When these are expressed in terms of body surface area (Table III) the values are 790 ± 20 liters/day per m^2 and 1,320 ± 70 liters/day per m^2. Again the estrone values are significantly higher than the estradiol values whether or not they are corrected for body surface area ($P < 0.01$). The MCR for both steroids was determined in six subjects on separate occasions, as shown in Table IV, and all these subjects showed a higher MCR (liters of plasma/day or liters of plasma/day per m^2) for estrone than for estradiol.

Comparison of results in the two phases of the cycle (Table V) shows that the mean plasma MCR of 1,360 ± 60 liters/day for estradiol in nine females in the follicular phase of the cycle is not signficantly different ($P > 0.10$) from the mean values of 1,280 ± 70 liters/day in six females in the luteal phase. Similarly the mean plasma MCR of 2,000 ± 130 liters/day for estrone in seven females in the follicular phase is not significantly different ($P > 0.10$) from the mean value of 2,350 ± 160 liters/day in five females in the luteal phase.

The mean estrone plasma MCR's do not significantly differ between females and males ($P > 0.10$) whether measured in liters/day, or liters/day per m^2. The slight difference (14%) in the mean plasma clearance rate (liters/day) is elim-

inated when expressed in liters/day per m^2 (Table III). The mean estradiol plasma MCR values in females is significantly lower than the mean values in males ($P < 0.01$). This significant difference is maintained when the values are corrected for body surface area ($P < 0.01$) even though the difference between the mean values drops after correction from 30 to 20% (expressed in terms of the male clearance rate).

Whole blood MCR. The mean ratios between the whole blood and plasma MCR's were determined in four males for estradiol and in five males for estrone. These ratios were found to be 1.62 ± 0.05 and 1.53 ± 0.05 respectively. Similarly the mean ratios between whole blood and plasma MCR's determined for estradiol (eight females) and for estrone (seven females) were 1.72 ± 0.05 and 1.45 ± 0.05 respectively. The difference in the ratios between estrone and estradiol was significant in females but not in males. All the plasma MCR's were then multiplied by the appropriate ratio in order to give whole blood clearance rates.

Males. The mean whole blood MCR in 11 males is $3,060 \pm 160$ liters/day for estradiol and $3,880 \pm 220$ liters/day in nine males for estrone. When expressed in terms of body surface area, the mean values are $1,600 \pm 80$ liters/day per m^2 and $1,990 \pm 120$ liters/day per m^2 respectively. The values for estrone and estradiol are significantly different ($P < 0.01$) however they are expressed.

Females. The mean whole blood MCR in 13 females is $2,280 \pm 90$ liters/day for estradiol and $3,200 \pm 170$ liters/day in 11 females for estrone. When expressed in terms of body surface area, the mean values are $1,360 \pm 40$ liters/day per m^2 and $1,910 \pm 100$ liters/day per m^2. The values for estrone and estradiol are significantly different ($P < 0.01$) however they are expressed.

When the mean estrone values for males and females are compared, their difference calculated on the basis of liters/day is about 17% of the male value, which is just significant ($0.05 > P > 0.02$). The difference between the mean values expressed in liters/day per m^2 becomes much smaller, 4% of the male value, and is not significant ($P > 0.10$).

The mean estradiol MCR measured in liters/day is significantly lower ($P < 0.01$) in females than males, and the difference is 25% of the male value. When the mean values are expressed in liters/day per m^2, the difference is only 15% of the male value. This difference, however, is significant ($P < 0.01$).

Conversion ratio. The conversion ratio of estrone to estradiol is obtained as follows: $C_{BB}^{1,2} = x^2/x^1$ [x^1 = plasma radioactive concentration of estrone as precursor; x^2 = plasma radioactive concentration of estradiol as product]. The conversion ratio of estradiol to estrone is obtained as follows: $C_{BB}^{2,1} = z^1/z^2$ [z^2 = plasma radioactive concentration of estradiol as precursor; z^1 = plasma radioactive concentration of estrone as product]. Three females and one male were infused with both estrone and estradiol. Following these infusions, both whole blood and plasma concentration of radioactivity in precursor and product were determined. The ratios between whole blood and plasma values were 1.01 ± 0.10 and 1.04 ± 0.10 for $C_{BB}^{1,2}$ and $C_{BB}^{2,1}$ respectively. Neither of these ratios differs significantly from 1.0 ($P > 0.10$), although theoretically they could differ.[6] Plasma values which were determined in all subjects will be used throughout.

The mean conversion ratios of estrone to estradiol (Tables II and IV) after infusion of estrone $C_{BB}^{1,2}$ are $6.9 \pm 0.2\%$ (nine males) and $6.5 \pm 0.6\%$ (10 females), which are not significantly different. For females there is no statistical difference between the mean values measured in the follicular and those in the luteal phases of the cycle ($5.8 \pm 0.5\%$ vs. $6.7 \pm 1.1\%$, $P > 0.10$).

The mean conversion ratios of estradiol to estrone (Tables II and IV) after infusion of estradiol are $11.3 \pm 0.9\%$ (11 males) and $11.0 \pm 1.0\%$ (13 females). These values are not significantly different in the two sexes. In females there is no statistical difference between the values for the follicular and luteal phases of the cycle ($11.9 \pm 1.3\%$ vs. $9.8 \pm 1.2\%$, $P > 0.10$).

$[\rho]_{BB}$ values (transfer constants) measured in plasma or blood after intravenous infusion. The

[6] e.g. $[\rho]_{BB}^{1,2} = \dfrac{MCR^2 \times x^2}{MCR^1 \times x^1} = \dfrac{x^2/z^2}{x^1/z^1}$

x^2/z^2 and x^1/z^1 will be equal for whole blood and plasma, if the radioactivity is in equilibrium, since the measurement is the ratio of isotopes in the same steroid. Therefore, the $[\rho]_{BB}$ value measured in whole blood or plasma should be equivalent, theoretically.

However, the conversion ratio e.g., $C_{BB}^{2,1} = z^1/z^2$, will not necessarily be equal when measured in whole blood or plasma if the distribution of estradiol and estrone into red cells is different.

TABLE VI

Per Cent of Radioactivity in Whole Blood Associated with the Red Cells and the Distribution Coefficient Red Cells and Plasma

No. of subjects	Sex	Steriod	Uptake into red cells*	Dist. Coeff. RBC plasma*
			%	%
(5)	Male	Estrone	19.8±1.5	28.3±2.7
(7)	Female	Estrone	14.1±1.9	24.0±3.8
(5)	Male	Estradiol	15.3±4.0	16.1±3.8
(7)	Female	Estradiol	2.2±0.6	3.4±0.9

* All values ± SE.

$[\rho]_{BB}$ (transfer constant) can be defined (3) as that fraction of the precursor which is converted to a product when the precursor is infused, and when the product is measured in the peripheral blood. The $[\rho]_{BB^{1,2}}$ value for the conversion of estrone to estradiol in blood is calculated from the rate of infusion of estrone [Rx^1 = plasma radioactive concentration of estrone (x^1) multiplied by the MCR of estrone (MCR^1)] and the rate of appearance of radioactive estradiol [Rx^2 = plasma radioactive concentration of estradiol (x^2) multiplied by the MCR of estradiol (MCR^2)].

Thus:

$$[\rho]_{BB^{1,2}} = \frac{Rx^2}{Rx^1} = \frac{MRC^2 \times x^2}{MCR^1 \times x^1}.$$

Similarly the $[\rho]_{BB^{2,1}}$ value for conversion of estradiol to estrone in blood is calculated as follows:

$$[\rho]_{BB^{2,1}} = \frac{R_z^1}{R_z^2} = \frac{MCR^1 \times z^1}{MCR^2 \times z^2}.$$

The $[\rho]$ values are equal when measured in plasma and whole blood, as would be expected theoretically.[6] In three females and one male who received infusions of both estrone and estradiol, with determinations of precursor and product radioactivity in both whole blood and plasma, the ratios between blood and plasma values are 1.02 ± 0.09 and 1.08 ± 0.06 for $[\rho]_{BB^{1,2}}$ and $[\rho]_{BB^{2,1}}$ respectively. Neither of these ratios are significantly different ($P > 0.10$) from 1.0 and plasma values will be used throughout.

Males. Table II. From the data given previously: $[\rho]_{BB^{1,2}} = 5.0 \pm 0.3\%$ and $[\rho]_{BB^{2,1}} = 15.6 \pm 1.2\%$.

Females. As computed from previous data: $[\rho]_{BB^{1,2}} = 4.1 \pm 0.4\%$ and $[\rho]_{BB^{2,1}} = 17.3 \pm 1.7\%$. The $[\rho]_{BB^{1,2}}$ and $[\rho]_{BB^{2,1}}$ do not significantly differ between the sexes, $P > 0.10$ for both. The $[\rho]_{BB}$ values in females do not differ when measured in the follicular or luteal phases: $[\rho]_{BB^{1,2}}$ is 4.1 ± 0.5% and 3.6 ± 0.4%; $[\rho]_{BB^{2,1}}$ is 17.5 ± 2.4% and 17.3 ± 1.0% for the two phases respectively.

Uptake into red cells. (Table VI). When the MCR's were determined simultaneously in whole blood and plasma, the per cent of radioactivity of the infused steroid which was associated or taken up by the red cell in whole blood was determined by the following formula:

$$\text{Per cent taken up by RBC's} = \frac{X_B - X_P(1 - \text{Hct})}{X_B} \times 100\%,$$

where X_B = radioactivity as precursor in 10 ml whole blood

X_P = radioactivity as precursor in 10 ml plasma

Hct = hematocrit as determined by the Wintrobe method (21).

Males. 19.8 ± 1.5% of the estrone radioactivity and 15.3 ± 4.0% of the estradiol radioactivity were associated with the red cells in two groups of five males.

Females. 14.1 ± 1.9% of the estrone radioactivity was associated with the red cells in seven females. However, only 2.2 ± 0.6% of the estradiol radioactivity was associated with the red cells in 10 females, and this value was significantly lower than all other values, $P < 0.01$. Using both steroids, we studied four subjects, and in all four there was less radioactivity associated with the red cells with estradiol than with estrone.

Distribution coefficient. Another way of expressing the association of estrone and estradiol to red cells is by the distribution coefficient for effective concentration of radioactivity in red cells (radioactivity divided by volume) to plasma. This association can be calculated by the following formula:

$$\text{Dist. Coeff.} = \frac{\frac{X_B}{X_P} - (1 - \text{Hct})}{\text{Hct}} \times 100\%.$$

The values as shown in Fig. 1 and Table VI are as follows: In males estrone has a distribution

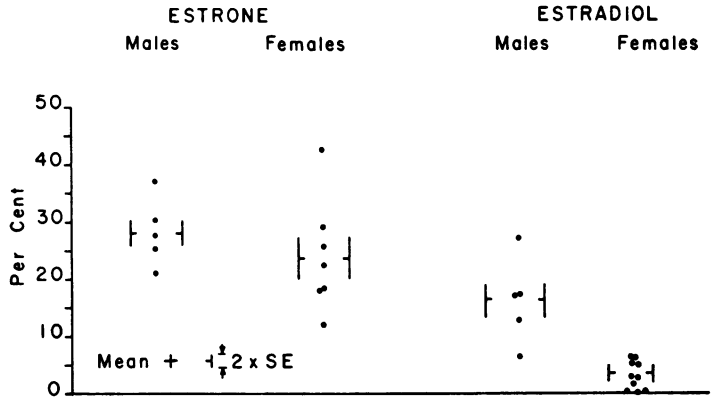

FIGURE 1 The distribution coefficient (effective concentration of steroid in RBC: Plasma) for estrone and estradiol in males and females.

coefficient of 28.3 ± 2.7% and estradiol 16.1 ± 4.0%. These values are significantly different ($0.02 > P > 0.01$). In females the distribution coefficient of estrone is 24.0 ± 3.8% and for estradiol it is 3.4 ± 0.9%. The last value is significantly lower than all the others ($P < 0.01$)

Following estradiol infusions into three females, blood was collected and centrifuged in the usual fashion at 4°C, and samples were also collected and centrifuged at room temperature. The red cell uptakes at 4° and 25°C were 0.0 and 0.0; 1.5 and 1.0; and 3.8 and 6.2% respectively for the three experiments and the corresponding distribution coefficients were 0.0 and 0.0; 2.3 and 1.5; and 6.0 and 10.8%. There was therefore no consistent difference between the values measured at 4°C and 25°C.

DISCUSSION

Two methods have been described in order to measure the metabolic clearance rate: the single injection and the continuous infusion techniques (22). We have used the continuous infusion technique throughout the study in order to determine MCR's and interconversions of estrone and 17β-estradiol in the circulation. While the single injection technique would have given us information concerning the volumes of distribution, it would have yielded imprecise information concerning the interconversions that occur between these two steroids.

Metabolic clearance rates have generally been expressed in terms of liters of plasma cleared per day (1–4). A correlation between metabolic clearance rate and body surface area has been noted however (13), and Horton and Frasier have shown that androstenedione clearances are similar in both adults and children (23) when expressed in terms of body surface area.

The mean values for plasma MCR of estrone in males and females are not significantly different whether or not they are expressed in terms of body surface. When the MCR is expressed in terms of whole blood, the difference between the mean estrone MCR in males and females is just significant ($0.05 > P > 0.02$) but when expressed in terms of body surface area the difference disappears.

The mean plasma and whole blood MCR's for estradiol in males are significantly greater than the respective MCR's in females ($P < 0.01$ for both), and these differences persist when the MCR's are expressed in terms of body surface area, although the differences become smaller when this correction is used.

We shall use the whole blood MCR expressed as liters/day per m^2 body surface area in our comparisons with hepatic blood flow.[7] However, in dis-

[7] To illustrate that $MCR_{whole\ blood}$ should be compared with hepatic blood flow rather than comparing MCR_{plasma} with hepatic plasma flow in order to assess splanchnic extraction (or extrasplanchnic metabolism) accurately, consider that, after a continuous infusion of a radioactive steroid, the subsequent constant radioactive concentration in the peripheral and afferent hepatic arterial blood are equal and are X_P in plasma and X_B in the hemolyzed whole blood. Then the total rate of clearance of

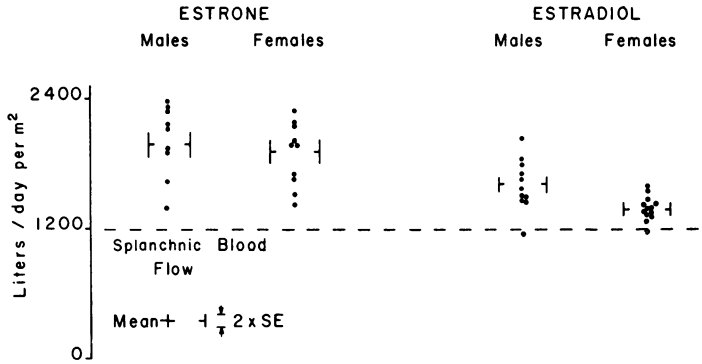

FIGURE 2 Comparison between metabolic clearance rates expressed in liters of whole blood/day per m^2 and estimated splanchnic blood flow in liters of whole blood/day per m^2.

cussing transfer constants, [ρ] values, we shall continue to use plasma values which are equivalent both theoretically and experimentally to whole blood values.[6]

As shown in Fig. 2, the whole blood MCR's of estrone expressed as liters/day per m^2 in both males and females (1990 and 1910 liters/day per m^2) are well above the estimated splanchnic blood flow of 1200 liters/day per m^2 (24). Since the splanchnic tissue can clear only that volume of blood which flows through it per day, it is apparent that at least 700 liters/day per m^2 must be cleared by extrasplanchnic tissue. Thus, at least 35% of the total estrone metabolism is extrasplanchnic.

radioactive steroid will be an equation: $MCR_{plasma} \times X_P = MCR_{WB} \times X_B$. Consider also a situation in which the splanchnic extraction of steroid is 100% and the extrasplanchnic metabolism zero. Then hepatic blood flow (HBF) $\times X_B$ will be the total rate of clearance of radioactive steroid. This rate will equal $MCR_{plasma} \times X_P$ or $MCR_{whole\ blood} \times X_B$ but will not necessarily equal hepatic plasma flow (HPF) $\times X_P$. Then $MCR_{whole\ blood} \times X_B = HBF_{whole\ blood} \times X_B$, and $MCR_{whole\ blood} = HBF$. In general if there is an uptake of steroids into the red cells, MCR_{plasma} will not equal HPF. Similarly it can be shown that, in general,

$$MCR_{whole\ blood} = HBF \times HE + ESC_{whole\ blood},$$

in which HE is the hepatic extraction of steroid as measured in whole blood, and $ESC_{whole\ blood}/MCR_{whole\ blood}$ is the proportion of the total metabolism which is extrasplanchnic. In previous estimates of the splanchnic extraction and the extrasplanchnic metabolism of androstenedione, testosterone (3), and aldosterone (22), the MCR_{plasma} was compared with HPF, and the HE and the ESC were slightly overestimated because of these considerations.

The clearance rate of estradiol in both sexes is also above the estimated splanchnic blood flow, but the portion of metabolism which is extrasplanchnic is less for estradiol than for estrone. At least 25% of the total estradiol metabolism in males, and at least 15% of the total in females is extrasplanchnic. It must be realized, however, that these estimates of extrasplanchnic metabolism are minimal values and presuppose an hepatic extraction of 100% for the respective estrogen. If the hepatic extraction of either steroid is less than 100%, then the estimated amount of extrasplanchnic metabolism will increase proportionately.

When the MCR is below splanchnic blood flow, as for testosterone and cortisol, it is evident that the estimated maximal splanchnic extraction of the steroid will be less than 100% (splanchnic extraction $\leq$ MCR $\div$ splanchnic blood flow $\times$ 100). When the MCR is above splanchnic blood flow, as in our estrone and estradiol studies, no real estimate of the splanchnic extraction of the infused steroid can be made.

In females the phase of the cycle does not seem to influence the MCR's of estrone or estradiol. We did not specifically study the time of ovulation, when estrogen secretion is assumed to be at a peak level (25), but if ovulation does have an effect on the clearance rate, it is apparently not sustained throughout the latter part of the cycle.

In both sexes the clearance rate for estrone is significantly above that of estradiol. Similarly, the 17-ketone, Δ^4-androstenedione has a higher clearance rate than the 17-hydroxy compound, testosterone. Testosterone is bound to a plasma protein

other than albumin (26), presumably a globulin, whereas androstenedione is bound less strongly (27). Recently Rosenbaum, Christy, and Kelly (28) have reported that estradiol may also be bound to a globulin and estrone less strongly. The greater binding to a plasma protein other than albumin (22) may then be responsible for the lower clearance rates of the 17-hydroxy compounds as compared to the 17-ketone compounds. On the other hand, apart from considerations of differential binding, the 17-ketone group or an unhindered ketone group as in progesterone (2) may render the steroid more liable for metabolism, particularly extrasplanchnic metabolism. Cortisol in the hepatectomized dog is metabolized mainly by reduction of the 20-ketone position (29).

Bardin and Lipsett (13) and Southren and coworkers (30) have reported that the plasma clearance rate of testosterone is lower in females than in males. Although, in the light of the considerations discussed in this paper, it would be more meaningful to compare whole blood clearance rates, the magnitude of the effect is such that a significant difference in the whole blood values between sexes for testosterone would also be expected. The binding of testosterone to proteins in plasma of females is rather higher than the binding to proteins in plasma of males, according to the data of Pearlman and Crépy (26). Similarly, our data could be explained by the fact that estradiol is more strongly bound to proteins in the plasma of females than males, as suggested by Tavernetti et al. (31).

Uptake of estrone by red cells has been noted and studied by several groups (7, 8). Migeon, Wall, and Bertrand (8) reported that 15–20% of radioactive estrone in the blood was associated with the red cells after injection of ^{14}C-estrone. This same distribution between plasma and red cells was also demonstrated in vitro again using ^{14}C-estrone (32, 33). Wall and Migeon noted that the isotopic estrone could be washed off the red cells with saline solution as well as with solutions of plasma proteins (32), and they also believed that estrone was adsorbed on the surface of the cell.

In both sexes we find that, following an infusion of ^{3}H-estrone, about 15–20% of the estrone radioactivity in whole blood is associated with the red cells. In males, similarly, 15% of the estradiol radioactivity in whole blood is found in association with the red cells. In females, however, only 2% of the estradiol radioactivity in the whole blood is associated with the red cells. It should be noted that, in the majority of our experiments, the blood was drawn into syringes, transferred to chilled tubes and then centrifuged at a cold temperature. This was done to minimize the possible interconversions that might occur in vitro (33) and cause errors in determinations of $[\rho]_{BB}$ values. This treatment might have had an effect on the distribution of ^{3}H-estrogen, since the affinity of some proteins for steroids is greater at 4°C than at 37°C (34). However, in the three experiments in which comparison was made between blood processed at 4°C and at 25°C, no trend in the uptake values or distribution coefficients could be shown. This would suggest that these values are not markedly altered by the exposure to 4°C. Neither does such exposure influence the MCR's, C_{BB} as determined in whole blood or $[\rho]_{BB}$, or production rates determined in plasma or whole blood.

The low level of estradiol radioactivity in females associated with the red cells is reflected by its very low distribution coefficient between red cells and plasma (Fig. 1). This fact suggests that there may be some degree of binding of estradiol, at least in females, to a protein other than albumin in plasma, although the possibility that this binding might be due to red cell characteristics cannot be excluded from the data presented here.

Beer and Gallagher (35) showed that estrone and estradiol were interconvertible in the body, and that the same pattern of urinary metabolites appeared after injection or ingestion of either steroid. Fishman, Bradlow, and Gallagher (36) showed that the pathway of estradiol to estriol, a major urinary metabolite, was through estrone, and that interconversion of estradiol and estrone was strongly in favor of estrone, since the reaction of estrone to estradiol occurred less readily.

As defined by Gurpide, MacDonald, Vande Wiele, and Lieberman (37), the $[\rho]$ value, or transfer constant is the fraction of the infused steroid which is converted to another steroid. They also devised experimental means by which this transfer constant could be measured, using the isotopic ratios that appeared in urinary metabolites. When this technique was applied to estrone and estradiol, values of 83 and 66% were obtained for $[\rho]^{2,1}$ and $[\rho]^{1,2}$ respectively (9). Barlow, (11)

using similar techniques, obtained values of 90 and 35% for the same conversions. Our $[\rho]_{BB}^{2,1}$ and $[\rho]_{BB}^{1,2}$ values are far lower, 15 and 5% respectively. We used calculations similar to those which were used by Horton and Tait (3) to derive the interconversion rates of androstenedione and testosterone when measured in blood. This marked discrepancy between the values obtained from urinary metabolites and from blood radioactivity can occur because the conversions measured in urine largely take place in a compartment, or compartments, not in equilibrium with the blood pool of the free steroids. Thus, the precursor is converted to the product steroid which is then, to an appreciable extent, further metabolized without contributing to the circulating blood pool of the product. Such a conversion would contribute to the $[\rho]$ value as determined from a urinary metabolite, but not necessarily to the $[\rho]_{BB}$ value as determined by us in blood. Similar conclusions about the compartments in which these conversions occur were also reached by Lipsett and coworkers (4), and Hembree, Bardin, and Lipsett (38).

This same difference between $[\rho]$ values determined in plasma and in urine has also been noted for androstenedione and testosterone (3, 4, 6). It was further observed that the conversions contributing to the $[\rho]_{BB}$ values in plasma occurred mainly extrasplanchnically (3), and that the products of the conversions occurring in the splanchnic area were metabolized before entering the blood (3). A similar situation might exist for estrone and estradiol, and the extrasplanchnic metabolism noted for each steroid could be responsible for the conversion measured in the blood. In females, at least, the $[\rho]_{BB}^{2,1}$ value of 17% agrees closely with the minimal figure for extrasplanchnic metabolism, which suggests that most of the extrasplanchnic metabolism can be accounted for by this reaction, if the splanchnic extraction is 100%. Side reactions of estradiol in males and of estrone in males and females must occur, other than those reactions to the respective products. These side reactions would explain the high degree of extrasplanchnic metabolism, unless the product is extracted in extrahepatic tissues.

We stated earlier that when the MCR of a steroid was greater than estimated splanchnic blood flow, no estimate of the splanchnic extraction of that steroid could be made. This statement applies to secreted steroid entering the splanchnic tissue from its afferent circulation. But, using the $[\rho]$ values obtained from urinary metabolites, and the $[\rho]_{BB}$ values obtained in blood, it is possible to estimate the minimum splanchnic extraction for steroids formed from the precursor directly in the splanchnic tissues. If the splanchnic tissues were responsible for the entire conversion of estrone to estradiol, and if the $[\rho]$ value from the urinary metabolites were 50% [the mean of values quoted by Vande Wiele (8) and Barlow (9)], then only 10% of this value would escape into the blood, since the $[\rho]_{BB}^{1,2}$ value is 5%. Thus the hepatic extraction for estradiol formed from estrone in the splanchnic tissues is at least 90%. If some part of the $[\rho]_{BB}^{1,2}$ value results from extrasplanchnic conversion, then the calculated splanchnic extraction for estradiol formed in the splanchnic bed will rise proportionately. Similarly, the splanchnic extraction for estrone formed from estradiol in the splanchnic bed must be at least 85% (mean urinary $[\rho]^{2,1} = 85\%$; $[\rho]_{BB}^{2,1} = 15\%$). Again, this is a minimal figure which assumes no extrasplanchnic conversion. If the situation on the site of the interconversion of estradiol and estrone is similar to that of androstenedione and testosterone, the correct value of splanchnic extraction will be much greater. It should be noted that in the case of testosterone it has been concluded that the extraction of testosterone formed from androstenedione may be greater than the testosterone entering the splanchnic circulation (3).

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