Plasma Precursors of Estrogen. I. Extent of Conversion of Plasma Δ^4-Androstenedione to Estrone in Normal Males and Nonpregnant Normal, Castrate and Adrenalectomized Females

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ABSTRACT. After the intravenous administration of tracer doses of 4-^{14}C-Δ^4-androstenedione +6,7-^{3}H-estrone (or 6,7-^{3}H-estradiol) to normal adult males, and normal, castrate and adrenalectomized young adult females, radioactive urinary metabolites of estrogen were isolated from 72-hr urine collections. In each subject studied a fraction of the administered 4-^{14}C-Δ^4-androstenedione was metabolized in the same manner as was the injected tritium labeled estrone. The extent of conversion of 4-^{14}C-Δ^4-androstenedione to the product hormone, estrone, was calculated from the relationship between the ^{3}H/^{14}C ratio of the administered tracers and that of the isolated urinary estrone. In the subjects studied an average of 1.3% of Δ^4-androstenedione (range 1.0–1.7%) was converted to estrone. These results indicate that in the young adult female an "extraglandular" source of estrone of approximately 44 μg/day may arise from plasma borne Δ^4-androstenedione. In the adult male, 18 μg of estrone /day can be expected to originate from plasma Δ^4-androstenedione. (*J Clin Endocr* **27:** 1103, 1967)

DEFINITIVE evidence for the conversion of parenterally administered testosterone[2] to naturally occurring estrogens in the human has been accumulated (1–4). But with respect to the physiologic significance of estrogen production from endogenous testosterone or other circulating C_{19} precursors, little or no information is available concerning the following: 1) the extent of conversion of each circulating C_{19} precursor to estrogen; 2) the biosynthetic nature of this conversion, specifically the estrogenic hormone(s) initially produced; 3) the quantity of estrogen which arises by this mechanism; 4) the availability to the general circulation of the estrogenic hormone(s) derived from the utilization of circulating precursors; and 5) the site(s) of aromatization of the plasma borne precursors.

The purpose of the present report is to describe our findings relative to the extent of conversion of circulating Δ^4-androstenedione to estrone in nonpregnant subjects. In preliminary studies (unpublished results) it was determined that, of the four endogenously produced, possible estrogen precursors (testosterone, Δ^4-androstenedione, dehydroisoandrosterone and dehydroisoandrosterone sulfate), Δ^4-androstenedione was converted to estrogen to a greater extent than the other three. While Δ^4-androstenedione is five times more efficient than any of the other three in the female, it is three times more efficient in the male.

The results of this study demonstrate that an average of 1.3% (range 1.0–1.7%) of intravenously administered 4-^{14}C-Δ^4-

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[2] The following trivial names and abbreviations have been used in this text: Δ^4-androstenedione (Δ^4-A), androst-4-ene-3,17-dione; dehydroisoandrosterone, 3β-hydroxyandrost-5-en-17-one; dehydroisoandrosterone sulfate, 17-oxo-androst-5-en-3β-yl-sulfate; estrone (El); 2-methoxyestrone, 2-methoxy-3-hydroxyestra-1,3,5(10)-trien-17-one; 16-epiestriol, estra-1,3,5(10)-trien-3,16β,17β-triol; 16-OH-androstenedione, 16α-hydroxyandrost-4-ene-3,17-dione; 16-OH-testosterone, 16α,17β-dihydroxyandrost-4-en-3-one.

androstenedione is converted to the product hormone, estrone, in the normal nonpregnant subjects. Further, the same extent of conversion of 4-^{14}C-Δ^4-androstenedione to estrone occurs in the adrenalectomized female and in the oophorectomized female. Thus, an "extraglandular" source of estrogen of potentially sizable magnitude may arise from plasma borne C_{19} steroids, particularly from plasma Δ^4-androstenedione.

Materials and Methods

Experimental design. In order to determine the extent of conversion of a circulating C_{19} precursor to an estrogenic hormone, the technique of the "*in vivo* internal standard" has been used (5–8). A tracer dose of 6,7-^{3}H-estrone and 4-^{14}C-Δ^4-androstenedione is simultaneously administered intravenously to the test subject. All urine excreted for the succeeding 72 hr is collected. From each subject's 72-hr urine collection, estrone, estradiol and estriol (and in some studies other estrone metabolites) are isolated. The extent of conversion of the administered C_{19} estrogen precursor, 4-^{14}C-Δ^4-androstenedione, to the product hormone, estrone, is calculated as follows:

$$\frac{R^{I}_{E1-{}^3H}}{(R^{I}_{\Delta^4\text{-}A\text{-}{}^{14}C})\ (\text{fraction } \Delta^4 - A - {}^{14}C \rightarrow E1)} = {}^3H/{}^{14}C \text{ ratio urinary E1}$$

or,

$$\%\ \Delta^4 - A - {}^{14}C \rightarrow \text{Estrone} = \left(\frac{R^{I}_{E1\text{-}{}^3H}}{R^{I}_{\Delta^4\text{-}A\text{-}{}^{14}C}} \div {}^3H/{}^{14}C \text{ ratio urinary E1}\right) \times 100,$$

where $R^{I}_{E1\text{-}{}^3H}$ = the amount of radioactivity (dpm ^{3}H) injected as 6,7-^{3}H-estrone; $R^{I}_{\Delta^4\text{-}A\text{-}{}^{14}C}$ is the amount of radioactivity (dpm ^{14}C) injected as 4-^{14}C-Δ^4-androstenedione.

By this technique, the calculated conversion of 4-^{14}C-Δ^4-androstenedione to estrone represents the fraction of the administered carbon-14 labeled tracer which is metabolized in the same manner as the administered tritium labeled estrone. It should be noted that this calculation does *not* represent the extent of conversion of the administered 4-^{14}C-Δ^4-androstenedione to urinary estrone; but rather, it reflects the extent of initial aromatization of Δ^4-androstenedione to the parent hormone, estrone. In a few studies 4-^{14}C-Δ^4-androstenedione was injected together with 6,7-^{3}H-estradiol.

Subjects. All subjects studied were ambulatory. The age of each subject is shown in the tables of results. The 2 castrate subjects had sustained total abdominal hysterectomy and bilateral salpingo-oophorectomy because of benign pelvic disease more than 1 yr prior to this study. The adrenalectomized subjects had undergone bilateral adrenalectomy because of Cushing's disease 5 and 8 yr before the present study. None of the subjects in the present study were receiving medications except the adrenalectomized patients, who were receiving 35 mg of cortisone acetate and 0.05 mg of 9α-fluorocortisol-21 acetate daily.

Tracer preparation and administration. The methods of purification of the 6,7-^{3}H-estrone and estradiol tracers have been described (9). The 4-^{14}C-Δ^4-androstenedione[3] (58 mc/mmole) tracer was purified by liquid-liquid partition column chromatography using celite as support for solvent system J (Table 1). Further purification was accomplished by paper chromatography using the solvent system O.

The purified isotope labeled tracers were administered intravenously as a single dose in 10 ml of 10% ethanol in normal saline or as a continuous infusion of 500 ml of 6% ethanol in 5% glucose through Teflon tubing during a 4–6 hr infusion period. In one study, PM-2, the tracers were administered in 12 divided doses. The purpose of this study was to minimize the mass of Δ^4-androstenedione administered at any given time. A total dose of 1.5 μc of 4-^{14}C-Δ^4-androstenedione (8 μg) and 0.56 μc of 6,7-^{3}H-estrone was administered over a 3-day period. Each injection, containing 0.67 μg of Δ^4-androstenedione, was spaced 4 hr or more after the preceding. Urine was collected from the first injection until 48 hr following the last injection.

Urine collection and processing. All urine excreted by each test subject for 72 hr (96 hr in PM-2) following the beginning of the continuous infusion or from the time of the "single shot" administration of tracers was pooled. Eighty per cent of the 72-hr urine pool (except PM-2 in which 100% was used) was adjusted to pH 5.0 with 50% H_2SO_4, then diluted by 5% with sodium acetate buffer (1M). One million units of β-glucuronidase (Ketodase) was added to the acidified, buffered urine prior to incubation for 72 hr at 37 C. The liberated steroids were extracted from the urine with

[3] The 4-^{14}C-Δ^4-androstenedione used in these studies was prepared from 4-^{14}C-testosterone by oxidation with *t*-butyl chromate according to the method of Menini and Norymberski (13).

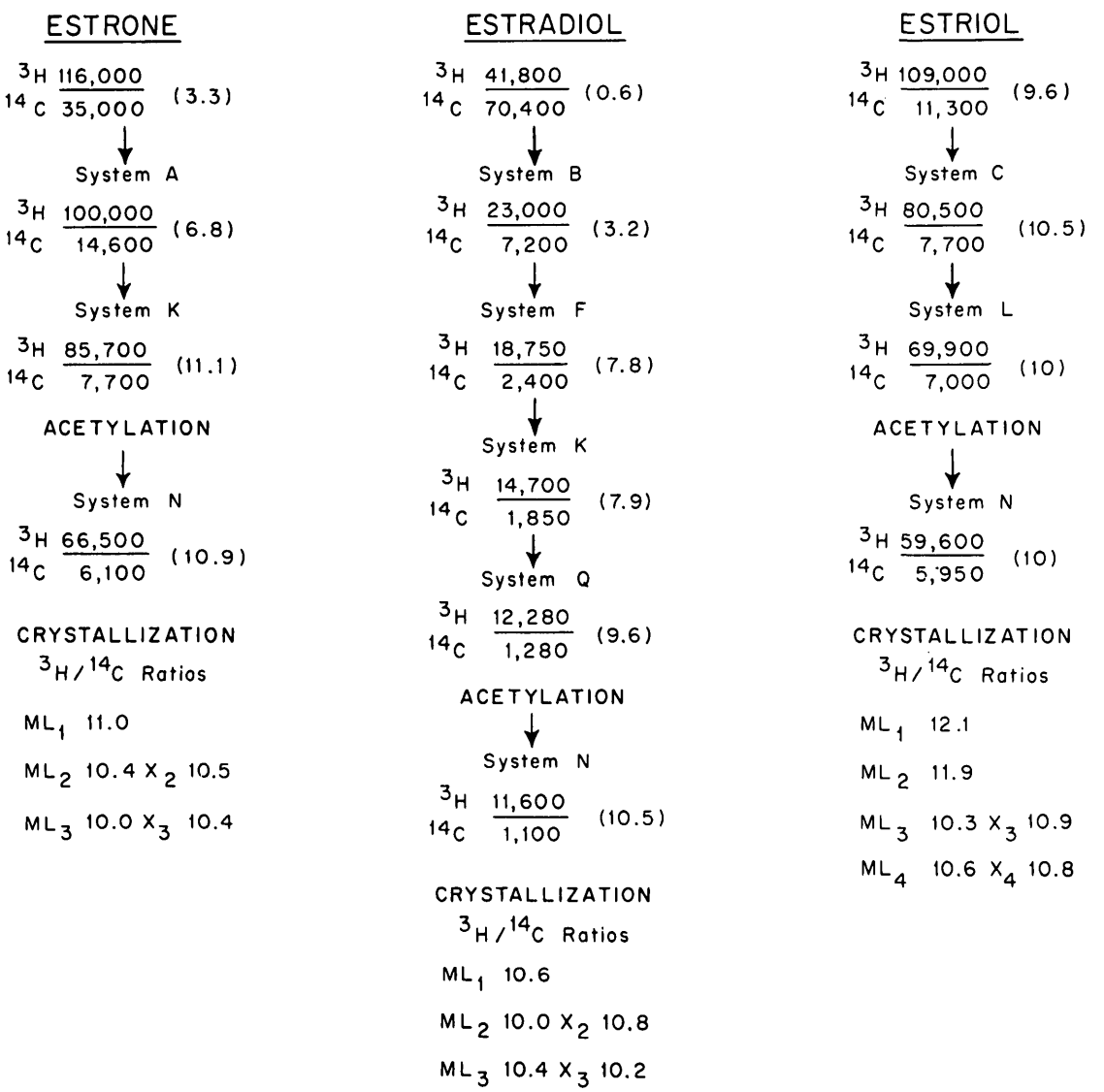

FIG. 1. Step by step purification following initial gradient elution partition chromatography of urinary estrogens from subject BK. The tracer dose used in this study was 8.5 μc of 4-^{14}C-Δ^4-androstenedione + 3.2 μc of 6,7-^{3}H-estrone. The ^{3}H and ^{14}C radioactivity and the ^{3}H/^{14}C ratios, in parentheses, at successive stages of purification are actual assay data observed in cpm. ML = mother liquor; X = crystals.

ethyl acetate. A "phenolic" extract was prepared as previously described (9).

In one study, performed in subject WC, multiple metabolites of estrone were isolated and their ^{3}H/^{14}C ratios were determined. In this experiment the urine was first treated with β-glucuronidase as described above. Following extraction of the steroids liberated by β-glucuronidase, the aqueous residue was adjusted to pH 1.0 with 50% H_2SO_4. Twenty g of NaCl was added to each 100 ml of the acidified aqueous residue before extraction with tetrahydrofuran according to the solvolysis technique of Burstein and Lieberman (10). Those phenolic steroids extracted after glucuronidase treatment and those obtained following solvolysis of the aqueous residue were separately purified by the techniques described below.

Isolation of urinary estrogens. Each urinary extract was initially subjected to celite partition chromatography, utilizing ethylene glycol as stationary phase and isooctane containing progressively increasing quantities of ethyl

TABLE 1. Chromatographic systems

Designation	Type*	Solvents	Volume ratio of solvents	Reference
A	Col.	Isooctane:*t*-butanol:methanol:water	(500:200:180:120)	9
B	Col.	Isooctane:*t*-butanol:methanol:water	(500:200:160:140)	9
C	Col.	Isooctane:*t*-butanol:methanol:water	(500:200:75:225)	9
D	Col.	Isooctane:*t*-butanol:methanol:water	(500:200:100:200)	9
E	Col.	*n*-Hexane:ethyl acetate:methanol:water	(1800:200:700:300)	11
F	Col.	*n*-Hexane:ethyl acetate:methanol:water	(1700:300:700:300)	11
G	Col.	Isooctane:ethyl acetate:methanol:water	(250:350:400:200)	—
H	Col.	*n*-Hexane:chloroform:methanol:water	(800:1200:700:300)	11
I	Col.	Isooctane:methanol:water	(1000:950:50)	—
J	Col.	Isooctane:methanol:water	(200:170:30)	—
K	TLC	Ether:methylene chloride	(8:92)	—
L	TLC	Ethyl acetate	—	9
M	TLC	Isooctane:ethyl acetate	(70:30)	—
N	TLC	Ether:methylene chloride	(4:96)	—
O	Paper	Isooctane:methanol:water	(100:90:10)	—
P	Paper	Decalin:nitromethane:methanol	(1000:500:500)	12
Q	Paper	Benzene:heptane:methanol:water	(700:300:800:200)	—

* Col. refers to liquid-liquid partition chromatography carried out on columns of celite. TLC refers to thin layer chromatography performed on 20 × 20 cm plates with layers of various thickness of Silica Gel G. Paper indicates descending paper chromatography on Whatman #1. These procedures have previously been fully described (9).

acetate as mobile phase. This system has previously been described in detail (9). Following the assay of radioactivity in 10% aliquots of each fraction obtained from the gradient elution chromatogram, the eluates containing estrone were combined, as were those containing estradiol, estriol and in one study 2-methoxyestrone and 16-epiestriol.

Further purification of estrone was accomplished by successive rechromatography utilizing systems A and K, and in some instances systems E and Q. Following purification of estrone, acetylation was carried out and the resulting acetate was cochromatographed with authentic estrone acetate in systems N and/or P. Additional nonradioactive estrone acetate was then added and the mixture was recrystallized until successive crystals and mother liquors contained identical $^{3}H/^{14}C$ ratios.

Estradiol isolated by gradient elution partition chromatography was further purified by successive rechromatography utilizing systems B and K, and in some instances systems F and Q were also used. Following these chromatographic steps the estradiol was acetylated and chromatographed together with authentic estradiol diacetate in system N. In some instances further purification of the estradiol diacetate was accomplished utilizing system P. Following chromatography, additional nonradioactive carrier estradiol diacetate was added and recrystallization was performed.

Estriol was further purified by successive chromatography utilizing systems C, L, and in some instances system H. The estriol was then reacted with pyridine and acetic anhydride and the resulting estriol triacetate was chromatographed together with authentic estriol triacetate in system N. The estriol triacetate was recrystallized with additional nonradioactive estriol triacetate.

In subject WC, 2-methoxyestrone isolated from the gradient elution chromatogram was further purified by successive rechromatography utilizing systems I, M and O. The 2-methoxyestrone was acetylated and then chromatographed with authentic 2-methoxyestrone acetate in systems N and P. 16-Epiestriol was also isolated in subject WC from the gradient elution chromatogram and further purified by successive rechromatography in systems D, G and L. The triacetate of 16-epiestriol was then prepared and chromatographed together with authentic 16-epiestriol triacetate in system N. The purified acetates were then recrystallized with authentic carriers as described above.

Results

Recovery and purification of radioactive urinary estrogen metabolites. A detailed account of the isolation of radioactive urinary estrogen metabolites and their content of ^{3}H and ^{14}C at various stages of purification in a typical study are shown in Fig. 1. It should be noted that this subject (BK) received the smallest tracer dose of any of the subjects studied except that in

TABLE 2. Tritium/carbon-14 ratio of urinary estrogens following the intravenous administration of 6,7-^{3}H-estradiol +4-^{14}C-Δ^4-androstenedione to normal men and women

Subject	Sex	Age	$^{3}H/^{14}C$ ratio (dpm) of injected tracers*	$^{3}H/^{14}C$ ratio (dpm) of urinary estrogens		
				Estrone	Estradiol	Estriol
SM	F	31	0.08-ci†	7.5	11	6.7
BC	F	27	0.15-ss	11	15	12
PM	M	34	0.15-ci	9.9	15	9.4
PS	M	37	0.16-ss	8.0	11	8.5
RA	M	37	0.16-ss	8.0	14	8.2

* Each subject received a tracer dose of between 9.9 and 14 μc of 4-^{14}C-Δ^4-androstenedione and between 1.06 and 2.17 μc of 6,7-^{3}H-estradiol.

† Method of tracer administration, ci = continuous infusion (4–6 hr) and ss = single shot. The tracer dose was administered to subject SM on the 25th day of a normal 28-day menstrual cycle and to subject BC on the 10th day of a normal 30-day menstrual cycle.

the special multiple dose study performed in PM-2. It can be seen that the quantity of radioactivity recovered in each metabolite was more than sufficient to establish its radiochemical homogeneity and therefore permit accurate determinations of $^{3}H/^{14}C$ ratios. Since these determinations were of primary importance to the objectives of this study, no attempt was made to achieve quantitative recoveries of urinary estrogens.

Radioactive urinary estrogens following the simultaneous administration of 6,7-^{3}H-estradiol plus 4-^{14}C-Δ^4-androstenedione. The results of the studies obtained following the simultaneous administration of tritium labeled estradiol and carbon-14 labeled Δ^4-androstenedione are shown in Table 2. Several findings are noteworthy. In each case there was conversion of the carbon-14 labeled Δ^4-androstenedione tracer to urinary estrogens, as indicated by the appearance of carbon-14 in the isolated estrogens.

It can be seen that in these studies the $^{3}H/^{14}C$ ratio of urinary estradiol was consistently higher than that of urinary estrone and estriol, which were similar. This result would be expected if the estrogen initially derived from circulating Δ^4-androstenedione was estrone rather than estradiol. This obtains since it is known from the studies of Fishman *et al.* (14) and of Gurpide *et al.* (15) that the metabolism of estradiol to estrone proceeds more rapidly than the metabolism of estrone to estradiol. In fact, these investigators have shown that only about 50% of estrone is metabolized via estradiol, whereas more than 90% of estradiol may suffer its metabolic fate via estrone. Accordingly, if estrone were the estrogen initially produced from 4-^{14}C-Δ^4-androstenedione, while the tritium labeled estrogen administered was estradiol, a higher $^{3}H/^{14}C$ ratio in urinary estradiol would be observed, as is shown in Table 2.

Urinary estrogen metabolites following the simultaneous administration of 6,7-^{3}H-estrone plus 4-^{14}C-Δ^4-androstenedione. The results obtained following the simultaneous administration of tritium labeled estrone plus carbon-14 labeled Δ^4-androstenedione are presented in Table 3. The $^{3}H/^{14}C$ ratios of urinary estrone, estradiol and estriol are very similar in these experiments. This contrasts with the findings reported above following the simultaneous administration of tritium labeled estradiol plus carbon-14 labeled Δ^4-androstenedione. These results indicate that the fraction of Δ^4-androstenedione converted to estrogen suffers a metabolic fate similar to that of estrone.

Also shown in Table 3 is the extent of conversion of circulating Δ^4-androstenedione to estrone occurring at the aromatizing site(s), as calculated from the $^{3}H/^{14}C$ ratio of urinary estrone and the $^{3}H/^{14}C$ ratio of the injected tracers. The extent of

TABLE 3. Tritium/carbon-14 ratio of urinary estrogens following the intravenous administration of 6,7-^{3}H-estrone + 4-^{14}C-Δ^4-androstenedione to normal males and to normal, castrate and adrenalectomized females

Subject	Sex	Condition	Age	$^{3}H/^{14}C$ ratio (dpm) of injected tracers†	$^{3}H/^{14}C$ ratio (dpm) of urinary estrogens			% conversion Δ^4-A→El*
					Estrone	Estradiol	Estriol	
HS	F	Normal	37	0.41-ci‡	41	42	47	1.0
MS	F	Normal	21	0.38-ci	31	35	39	1.2
HC	F	Normal	25	0.39-ci	32	33	30	1.2
AD	F	Normal	23	0.25-ci	15	—	16	1.7
CW	F	Castrate	29	0.12-ss	9.2	—	—	1.3
JF	F	Castrate	22	0.42-ss	38	34	37	1.1
SS	F	Adrenalectomized	25	0.27-ss	24	—	24	1.1
AR	F	Adrenalectomized	37	0.25-ss	15	13	15	1.7
WC	M	Normal	31	0.41-ci	35	34	35	1.2
BK	M	Normal	31	0.37-ss	24	24	25	1.5
SS	M	Normal	28	0.37-ss	28	—	30	1.3
TB	M	Normal	25	0.37-ss	28	26	27	1.3
PM-2	M	Normal	36	0.37-mi	25	25	26	1.5

* The extent of conversion of 4-^{14}C-Δ^4-androstenedione is calculated on the basis of the $^{3}H/^{14}C$ ratio of urinary estrone: % Δ^4-A→El = ($^{3}H/^{14}C$ ratio of injected tracers ÷ $^{3}H/^{14}C$ ratio urinary estrone) ×100. See text.

† Each subject (except WC and PM-2) received a tracer dose of between 8.5 and 26.2 μc of 4-^{14}C-Δ^4-androstenedione together with between 2.13 and 8.0 μc of 6,7-^{3}H-estrone. WC received 78.6 μc of 4-^{14}C-Δ^4-androstenedione + 32.5 μc of 6,7-^{3}H-estrone. PM-2 received a total dose of 1.5 μc of 4-^{14}C-Δ^4-androstenedione + 0.56 μc of 6,7-^{3}H-estrone.

‡ Method of tracer administration, ci = continuous infusion (4–6 hr); ss = single shot; mi = multiple injections. The tracer dose was administered to HS, MS, HC and AD on the 8th, 12th, 3rd and 5th day of normal 28-, 30-, 28- and 26-day menstrual cycles, respectively.

this conversion ranged from 1.0 to 1.7%. The extent of aromatization of plasma Δ^4-androstenedione in the oophorectomized and adrenalectomized subjects was the same as that found in the normal subjects in this study.

Particular note should be made of the results in subject PM-2, Table 3. In this study, following the administration of the isotope labeled tracers in small multiple doses, the extent of conversion of Δ^4-androstenedione to estrone was the same as that observed following the administration of the tracers as one single shot or by continuous infusion. Thus, within the range of doses of this study, the mass of Δ^4-androstenedione administered did not significantly affect its conversion to estrone.

$^{3}H/^{14}C$ ratios of multiple urinary metabolites following the simultaneous administration of 6,7-^{3}H-estrone + 4-^{14}C-Δ^4-androstenedione (subject WC). In subject WC (who received a large tracer dose by continuous intravenous infusion), multiple metabolites of estrone were isolated separately following glucuronidase treatment and following solvolysis. The results of this study are shown in Table 4. It can be seen that all of the isolated radioactive metabolites resulting from the simultaneous administration of tritium labeled estrone and carbon-14 labeled Δ^4-androstenedione had very similar $^{3}H/^{14}C$ ratios. In view of these results, it is unlikely that a significant amount of the administered 4-^{14}C-Δ^4-androstenedione was converted to estrogen via neutral pathways. Specifically, these results provide no evidence for the sequences, Δ^4-androstenedione⇄testosterone→estradiol; or Δ^4-androstenedione→16-OH-androstenedione ⇄16-OH-testosterone→estriol. Therefore, these findings provide additional evidence that the initial estrogen product derived from the aromatization of circulating Δ^4-androstenedione is solely estrone.

Discussion

Zondeck, in 1934, based on his observation that stallion urine contained large quantities of estrogen, while the urine of mares had little estrogen, stated, "I believe that the female hormone which is regularly present in the male organism represents a normal physiological product of the metabolism of the sex hormones, especially since—due to our present chemical knowledge (Butenandt, Marrian, Doisy)—a conversion of the male hormone into the female one appears to be quite possible" (16).

Evidence supporting Zondeck's deduction was reported by Steinach, Kun and Peczenik, who, in 1936, found that the administration of "male hormone" to intact and castrate male rats increased the excretion of "estrogenic" substances in their urine (17). In 1937, an additional report by Steinach and Kun described their finding of increased estrogen in the urine of six men who were treated with testosterone propionate (18).

Since these early studies, many reports have appeared describing the finding of increased urinary estrogens following the administration of various C_{19} compounds, principally testosterone. These have included studies in the intact and castrate, male and female, human and various experimental animals. The first definitive evidence for the increased estrogen excretion following C_{19} steroid administration being due to aromatization of the administered steroid was presented in 1956 by West and associates (19). These workers found estrone and estradiol in the urine of two adrenalectomized oophorectomized women following treatment with testosterone propionate. Prior to treatment, estrogen could not be detected in these subjects' urine. This study provided strong support for the thesis that the increased amounts of estrogen found in the urine after testosterone treatment arose from the conversion of testosterone to naturally occurring estrogens.

TABLE 4. Tritium/carbon-14 ratio (dpm) of multiple urinary metabolites of estrone in subject WC following the intravenous administration of 6,7-^{3}H-estrone +4-^{14}C-Δ^4-androstenedione. Injected $^{3}H/^{14}C$ ratio = 0.41.

	Glucuronosides	Sulfates
Estrone	35	37
Estradiol	34	—
Estriol	35	37
2-Methoxyestrone	33	34
16-Epiestriol	36	39

The tracers, 78.6 μc of 4-^{14}C-Δ^4-androstenedione +32.5 μc of 6,7-^{3}H-estrone, were administered by continuous intravenous infusion during a 6-hr period. The estrogen metabolites present in the urine as glucuronosides and as sulfates were separated by differential hydrolysis.

In 1962, Braun-Cantilo, LaRoche, Novitsky and Lawrence reported the finding of carbon-14 labeled estrogens in the urine of two women following the administration of carbon-14 labeled testosterone (1). Since then, reports by Fishman, Lipsett, Korenman and Davis (2) and by Ahmad and Morse (3) and Morse, Epstein, Fraw and Raheja (4) indicate that the administration of isotope labeled testosterone to normal men is followed by the appearance of radioactive estrogens in their urine.

Perhaps the most significant finding of the present study is the efficiency of the conversion of circulating Δ^4-androstenedione to estrone [avg 1.3% (range 1.0–1.7%)]. In a previous study it was found that the extent of conversion of plasma dehydroisoandrosterone sulfate to estrogen in nonpregnant subjects was much less than the conversion of Δ^4-androstenedione to estrone observed in the present study (5). The finding of very low conversions of dehydroisoandrosterone sulfate to estrogen has been confirmed by Mancuso *et al.* (20). Moreover, studies carried out in these laboratories have shown that Δ^4-androstenedione is more efficiently converted to estrogen than is circulating testosterone or unconjugated dehydroisoandrosterone (unpublished results).

The results herein reported also indicate that the "parent" estrogen derived from

circulating Δ^4-androstenedione is solely estrone. This is borne out by the fact that multiple urinary metabolites of estrone, both glucuronosides and sulfates, had the same $^3H/^{14}C$ ratio following the simultaneous administration of 6,7-^{3}H-estrone and 4-^{14}C-Δ^4-androstenedione but not following the administration of 4-^{14}C-Δ^4-androstenedione together with 6,7-^{3}H-estradiol. Therefore, the fraction of administered carbon-14 labeled Δ^4-androstenedione behaving as an estrogen suffered a metabolic fate identical with that of estrone in these studies.

The amount of carbon-14 labeled Δ^4-androstenedione administered in these experiments was chosen to insure, a) the maximum sensitivity for determining the extent of conversion of 4-^{14}C-Δ^4-androstenedione to estrone; and b) sufficient carbon-14 radioactivity in the urinary estrone metabolites to permit unequivocal determination of their radiochemical homogeneity. In view of the limited specific activity of available carbon-14 labeled tracers, the amount of radioactivity required to meet the stipulations cited above necessitated the administration of 41 to 128 μg of Δ^4-androstenedione. That this administered mass of Δ^4-androstenedione did not significantly influence the extent of its conversion to estrone is demonstrated by the fact that there was no difference in conversion whether the tracer was administered by "single shot," by continuous infusion during four to six hours, or by multiple injections of only 0.67 μg each at intervals of four hours or more.

The quantity of estrone derived from endogenously produced, circulating Δ^4-androstenedione depends ultimately not only on the extent of conversion of Δ^4-androstenedione to estrone, but also on the amount of Δ^4-androstenedione available for this process. In the normal female, plasma production rates of Δ^4-androstenedione of approximately 3.4 mg/day have been reported by Horton and Tait (21). Using this value as the plasma production rate of Δ^4-androstenedione, of which 1.3% is converted to estrone, a yield of 44 μg of estrone/day could be expected from the utilization of plasma borne Δ^4-androstenedione. In the normal adult male, producing 1.4 mg of Δ^4-androstenedione into the blood each day [Horton and Tait (21)], 18 μg of estrone /day should arise from plasma Δ^4-androstenedione. In preliminary studies in this laboratory, increased production rates of Δ^4-androstenedione have been observed in women with androgen producing ovarian tumors and in some women with chronic anovulation associated with sclerocystic ovarian disease. Increased production of Δ^4-androstenedione can be expected to yield proportionately larger quantities of "extraglandular" estrone, since the extent of conversion of circulating Δ^4-androstenedione to estrone under these circumstances is not reduced.

These studies provide little insight into the "physiological availability" of the estrone produced from circulating Δ^4-androstenedione. Specifically, the amount of estrone derived from circulating Δ^4-androstenedione which enters the circulation as the hormone, estrone, cannot be determined from these studies It is possible that the aromatization of Δ^4-androstenedione occurs at the same site(s) as that in which metabolism of estrone occurs. If this is true, a situation would exist in which part or all of the estrone derived from Δ^4-androstenedione could suffer immediate catabolism and conjugation without ever having entered the blood as estrone. This would represent a situation analogous to that described by Korenman and Lipsett in the case of testosterone derived from circulating Δ^4-androstenedione (22). However, in preliminary studies, measurements of the $^3H/^{14}C$ content of circulating estrone and estradiol following the continuous infusion of tritium labeled estrone and carbon-14 labeled Δ^4-androstenedione have indicated that more than 50% (and in one male sub-

ject, 100%) of the estrone arising from Δ^4-androstenedione does in fact enter the circulation.

The physiological and/or pathophysiological significance of "extraglandular" extrogen production from circulating C_{19} precursors as well as the site(s) of aromatization are yet to be determined However, the potential significance of a large extraglandular estrogen supply in terms of hypothalamic-pituitary-gonadal function and in terms of correctly delineating true glandular estrogen secretion is of obvious importance

The results of these studies are consistent with the following conclusions. 1) Intravenously administered 4-^{14}C-Δ^4-androstenedione is converted to estrogen. 2) The estrogenic hormone initially derived from circulating Δ^4-androstenedione is estrone. 3) In the adult, the fraction of circulating Δ^4-androstenedione converted to estrone at the aromatizing site, *i.e.*, the extent of initial aromatization, averages 1.3%. 4) The conversion of circulating Δ^4-androstenedione to estrone is not reduced in the absence of the ovary, the adrenal or the uterus. 5) Estrogen produced from plasma Δ^4-androstenedione may be expected to account for about 44 μg of estrone/day in the normal young adult female and for 18 μg of estrone production/day in the normal adult male.

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