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Research Article

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The hyperestrogenism was found to be the consequence of massive extraglandular conversion of plasma androstenedione to estrone. During a 6-mo period of study, the plasma production rate of androstenedione ranged from 1.2 to 1.6 mg/day. More than 55% of plasma androstenedione was metabolized by aromatization to estrone which, in turn, was extensively sulfurylated in the tissue sites of aromatization before its entry into the blood. Thus, estrone sulfate was the final product in the aromatizing sites, and the plasma production rate of estrone sulfate derived from plasma androstenedione was 782 μ g/24 h.

The extent of extraglandular conversion of plasma androstenedione to estrone measured in this boy was 50 times that observed in two normal prepubertal boys. Moreover, 94% of the extraglandular aromatization occurred in extrahepatic sites. The metabolic clearance rate of plasma androstenedione, 2,380 liters/day per m^2 , was markedly increased in [...]

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DAVID L. HEMSELL, CLARE D. EDMAN, JAMES F. MARKS, PENTTI K. SIITERI, and
PAUL C. MACDONALD

*From the Cecil H. and Ida Green Center for Reproductive Biology Sciences and the Departments of
Obstetrics and Gynecology and Pediatrics of the University of Texas Southwestern Medical
School, Dallas, Texas 75235*

A B S T R A C T This report describes the mechanism of origin and the quantity of estrogen produced in a prepubertal boy who developed severe feminization at 8 yr of age as the result of a heretofore undescribed metabolic abnormality. The clinical findings were gynecomastia and accelerated linear growth and bone maturation. At the time feminization developed, there were no signs of growth or development of the otherwise normal prepubertal male external genitalia or any increase of muscle mass that normally accompanies male puberty.

The hyperestrogenism was found to be the consequence of massive extraglandular conversion of plasma androstenedione to estrone. During a 6-mo period of study, the plasma production rate of androstenedione ranged from 1.2 to 1.6 mg/day. More than 55% of plasma androstenedione was metabolized by aromatization to estrone which, in turn, was extensively sulfurylated in the tissue sites of aromatization before its entry into the blood. Thus, estrone sulfate was the final product in the aromatizing sites, and the plasma production rate of estrone sulfate derived from plasma androstenedione was 782 μ g/24 h.

The extent of extraglandular conversion of plasma androstenedione to estrone measured in this boy was 50 times that observed in two normal prepubertal boys. Moreover, 94% of the extraglandular aromatization occurred in extrahepatic sites. The metabolic

clearance rate of plasma androstenedione, 2,380 liters/day per m^2 , was markedly increased in this boy. Approximately 1,500 liters of plasma androstenedione clearance was accounted for by extrahepatic, extraglandular aromatization. The fractional conversion of testosterone to estradiol, 0.16, was 50 times greater in this boy than that observed in normal young adult men. The total extent of aromatization of plasma prehormones was even greater in this boy inasmuch as evidence was obtained that aromatization of 16-hydroxysteroids, e.g. 16 α -hydroxy androstenedione and 16 α -hydroxy dehydroisoandrosterone (sulfate), resulted in estriol formation independent of estrone formation. Thus, extensive extrahepatic, extraglandular aromatization resulted in advanced feminization in this prepubertal boy by a previously undescribed metabolic abnormality.

INTRODUCTION

Feminization of prepubertal boys is extremely uncommon. Indeed, the occurrence of pure heterosexual precocity in young boys is so rare that it is usually ignored by authors discussing sexual precocity. Wilkins (1) stated that only adrenal tumors or exogenous estrogens, e.g. vitamins contaminated with estrogen, were responsible for feminization of the prepubertal male. Others have reported that tumors of the interstitial cells of the testes (2, 3) may cause feminization. In a review of six case reports of feminizing adrenal tumors in boys, Eberlein and Winter emphasized that each of these boys had also developed male pubertal sexual characteristics and (or) had moderate-to-high excretion rates of urinary 17-ketosteroids (4).

We have studied a prepubertal boy with advanced feminization, but no masculinization, that

Dr. Hemsell is a Lt. Col., Medical Corps, United States Air Force. The views expressed in this report are those of the authors and do not necessarily represent those of the U. S. Air Force.

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was associated with the formation of large amounts of estrone sulfate (E1S)¹ which arose by the extraglandular aromatization of plasma androstenedione to estrone. Sulfurylation of the estrone occurred within the tissue site(s) of aromatization before entry of the product estrogen, estrone sulfate, into blood. The cause of the massive extraglandular aromatase activity in this boy was not delineated by the results of this study. It is interesting to recall, however, that both extraglandular aromatization and sulfurylation are involved extensively in C₁₉-steroid metabolism in the fetus (5-10); however, the level of expression of these enzymes diminishes rapidly after birth. Thus, the continued expression of the fetal levels of both the aromatase and sulfokinase enzyme activities after birth could give rise to increased estrogen formation from plasma prehormones and give rise to feminization such as that observed in this child.

METHODS

Case report

The boy of this study was first seen by us when he was 10 yr 7 mo of age. At that time, his foster mother reported that he had experienced slowly progressive, nonpainful gynecomastia for 2 yr. Regrettably, the family, prenatal, labor, and delivery histories are unknown in that he is an adopted child; but his early growth, development, and general health were considered normal. Shortly after the onset of gynecomastia, his foster mother observed an accelerated rate of growth in this boy and that he was considerably taller than his peers. Small amounts of axillary hair were first noticed 2 mo before his visit to us. Sparse pubic hair was first observed 2 wk before that visit.

The subject was a tall (157 cm), thin (39.7 kg), prepubertal male with a highly pitched voice. His blood pressure and pulse rate were normal. There was no accentuation of male pubertal characteristics such as muscle mass definition, temporal hairline recession, acne, or facial, chest, or abdominal hair. There were a few dark brown axillary hairs. The breast areolae were enlarged and slightly pigmented. Breast tissue had developed symmetrically and corresponded to Stage IV female pubertal breast development according to Tanner (11) (Fig. 1). There was no galactorrhea. The fat distribution was typical of that of a prepubertal child. Sparse blackish-brown pubic hairs were present in a female configuration over the pubic region, consistent with Tanner Stage III pubertal female development. The circumcised penis (3 cm long and 1.5 cm wide) was normal for a prepubertal male. The urethral meatus was located normally in the glans penis. The scrotal skin was thin, pale, and without bifurcation. The testes were palpable in the inguinal canals but could be easily manipulated into the scrotum.

¹Abbreviations used in this paper: A, androstenedione (androst-4-ene-3, 17-dione); BB, blood to blood; BU, blood to urine; E1, estrone (estra-1,3,5(10)-triene-3-ol-17-one); E1S, estrone sulfate (estra-1,3,5(10)-triene-3-ol-17-one sulfate); E2, estradiol (estra-1,3,5(10)-triene-3,17 β -diol); E3, estriol (estra-1,3,5(10)-triene-3,16 α ,17 β -triol); k, conversion ratio; MCR, metabolic clearance rate; PPR, plasma production rate; PR, production rate; ρ , transfer constant; T, testosterone (17 β -hydroxyandrost-4-ene-3-one).

The right testis measured 2.5 \times 1.3 \times 1.0 cm, and the left was 1.0 \times 1.0 \times 1.0 cm. These measurements are similar to those of prepubertal males reported by Tanner (11) and by August et al. (12).

Laboratory evaluations performed before the time we saw this boy included measurement of urinary estrogens which were markedly elevated, plasma gonadotropins which were low, and urinary 17-hydroxycorticosteroid levels which were low. The urinary 17-ketosteroids were 5.3 mg/24 h. (These tests were performed by Bio-Science Laboratories of Van Nuys, Calif.) Cells from the buccal mucosa contained no identifiable sex chromatin masses; the karyotype from peripheral leukocytes was 46 XY. Routine hematologic studies, renal and hepatic function tests, serum electrolytes, urine analysis, skull, chest, and abdominal X ray, including an intravenous pyelogram, were normal. Bone age evaluation by X-ray examinations of the wrists and hands was found to correspond to that of a male 15-15 $\frac{1}{2}$ yr of age according to the standards of Pyle et al. (13). Brain and liver scans were normal. An abdominal aortogram was normal except for an area of suspicious vascularization in the left adrenal gland.

After these data were assembled, we concluded that this boy had a small feminizing adrenal tumor, probably on the left side, and elected to perform an exploratory laparotomy. When the initial exploration of the abdominal viscera and adrenals failed to reveal a tumor, the peritoneum was incised over each adrenal gland to allow direct inspection. Both adrenals were found to be normal. The retroperitoneal areas were also examined carefully but no abnormality was found. During surgery, blood was obtained from each adrenal vein, the inferior vena cava near the junction of the common iliacs, and from the left antecubital vein for measurement of plasma estrogen concentrations. Estrone (E1) concentrations in these plasma samples were 233, 122, 241, and 138 pg/ml from the left and right adrenal veins, the inferior vena cava, and the antecubital vein, respectively. There was no detectable estradiol (E2) in the adrenal vein bloods; however, estradiol levels of 28 and 32 pg/ml of plasma were found in caval and antecubital veins, respectively. These measurements were performed by the radioimmunoassay technique described by Mikhail et al. (14).

The postoperative course was uncomplicated, and he was discharged to outpatient care for further metabolic studies. The plasma concentrations of several C₁₈ and C₁₉ steroids, gonadotropins, and their relationship to body growth during a 2-yr period are presented in Table I.

Experimental design and tracer administration

Inasmuch as an adrenal or testicular source of estrogen secretion was not found in this boy with advanced feminization, we sought to ascertain if sufficient estrogen formation were occurring by extraglandular aromatization of a plasma prehormone(s) to cause the observed physical and laboratory findings, or alternatively, if there were an unknown source of direct estrogen secretion. Thus, we set out to measure total estrogen production and to quantify that portion of the total production that could be attributed to extraglandular formation from plasma prehormones.

The theoretical considerations upon which the experimental design of these studies was based have been described (15). As data accrued, the presumed physiological model was altered to include the metabolic sequence depicted in Fig. 2. It should be emphasized that the transfer constant of conversion of plasma androstenedione to estrone measured in this study, $[\rho]_{BU}^{AE1}$, represents the summa-

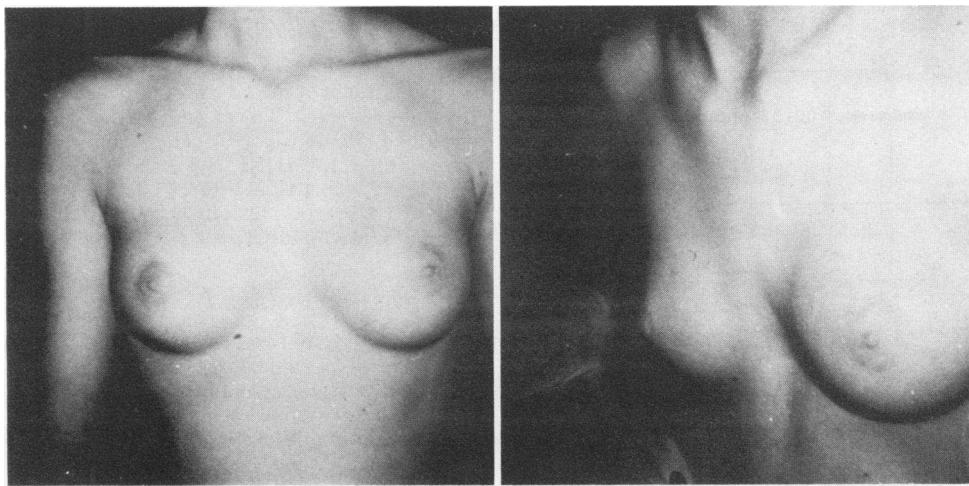


FIGURE 1 (Left) Frontal view of breast development in the prepubertal boy with heterosexual precocity. (Right) Lateral view.

tion of all pathways of conversion of plasma androstenedione to estrone. Indirect pathways, computed in this value, may include those in which androstenedione was converted to estrone and then to estrone sulfate in the tissue and released into blood as both estrone and estrone sulfate. Evidence will be presented to support the conclusion that the primary estrogen formed in this boy was estrone sulfate which arose by the extraglandular aromatization of plasma androstenedione giving rise to estrone which was sulfurylated before entry into blood from the aromatizing tissue sites.

Study I. Measurement of the production rate of estrone (PR-E1) and the transfer constant of conversion of plasma androstenedione (A) to estrone, ($[\rho]_{BU}^{AE1}$). In this study, performed when the subject was 10 yr 9 mo of age, the tracers $[6,7\text{-}^3\text{H}]E1$ and $[4\text{-}^{14}\text{C}]A$ were dissolved in 10 ml of normal saline containing 8% ethanol and injected intravenously as a bolus. Urine was collected for the next 72 h. The $[\rho]_{BU}^{AE1}$ and PR-E1 were computed from the $^3\text{H} : ^{14}\text{C}$ ratio and the specific activity, respectively, or urinary estrone

isolated after β -glucuronidase hydrolysis by methods previously described (16).

Study II. Measurement of the production rate of estradiol (PR-E2) and the transfer constant of conversion of plasma testosterone (T) to estradiol $[\rho]_{BU}^{TE2}$. In this study, performed when the subject was 10 yr 10 mo of age, $[6,7\text{-}^3\text{H}]E2$ and $[4\text{-}^{14}\text{C}]T$ were injected intravenously as a bolus. Urine was again collected for 72 h. The $[\rho]_{BU}^{TE2}$ and PR-E2 were computed from the $^3\text{H} : ^{14}\text{C}$ ratio and the specific activity, respectively, of urinary estradiol (glucuronoside). The $[\rho]_{BU}^{TE2}$ value represents the sum of all routes of conversion of plasma testosterone to estradiol. This includes the conversion of testosterone to estradiol directly as well as by indirect pathways, such as plasma testosterone \rightarrow plasma androstenedione \rightarrow estrone \rightarrow estrone sulfate \rightarrow estradiol sulfate \rightarrow estradiol.

Study III. Measurement of the metabolic clearance rate (MCR) and plasma production rate of androstenedione (PPR-A); the transfer constant of conversion of plasma androstenedione to plasma estrone, ($[\rho]_{BU}^{AE1}$); the trans-

TABLE I
Plasma Concentrations of Androgens, Estrogens, and Gonadotropins

Age yr/mo	Height cm	Weight kg	DHA ng/ml	A ng/ml	T ng/ml	E1 pg/ml		LH* mIU/ml	FSH† mIU/ml
						E2 pg/ml	PPR-A ng/ml		
10/11§	157	39.7	0.53	0.44	0.03	138	28	5	<6
11/3¶	158	41.9	—	0.56	0.36	178	15	—	—
11/6	159	41.0	1.9	0.10	0.68	82	8	<5	<5
11/9	160	44.0	1.0	0.36	0.71	266	15	<5	<5
12/0	160	51.5	0.95	0.44	0.70	427	51	<5	<5
12/3	160	52.5	1.9	0.46	1.4	558	73	<5	<5
12/6	161	51.0	0.84	0.68	4.1	370	50	<5	<5
12/9	163	48.9	—	0.44	3.9	382	25	<5	<5
13/0	163	51.6	—	0.51	2.7	545	59	<5	<5

* LH, luteinizing hormone.

† FSH, follicle-stimulating hormone.

§ Plasma concentrations used in Study III.

¶ Plasma concentrations used in Study IV.

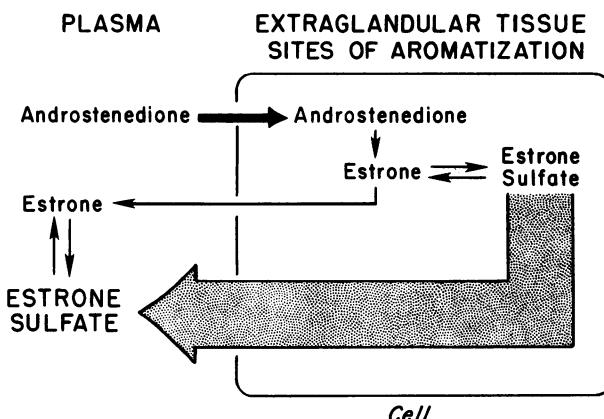


FIGURE 2 Possible mechanisms of extraglandular estrogen formation in a prepubertal boy with feminization.

splanchnic extraction and conversion of androstenedione to estrone; and the conversion ratio of plasma [^{14}C]A to [^{14}C]T, $([\text{k}]_{\text{BB}}^{\text{AT}})$ (17). The high plasma estrone concentrations found during surgery together with the data obtained from the first two studies led to Study III. This study was performed when the subject was 10 yr 11 mo of age and was designed to measure the following: (a) the daily plasma production rate of androstenedione (PPR-A); (b) the transfer constant of conversion of plasma androstenedione to plasma estrone ($[\rho]_{\text{BB}}^{\text{AE1}}$); (c) the total estrone production rate (computed from the specific activity of urinary estrone [glucuronoside]) and to compare this value to that portion of estrone computed to have arisen by the extraglandular aromatization of plasma androstenedione, i.e. does $\text{PR-E1}_A = \text{PPR-A} \times [\rho]_{\text{Bu}}^{\text{AE1}}$? (d) the conversion ratio of androstenedione to testosterone in plasma ($[\text{k}]_{\text{BB}}^{\text{TA}}$); (e) the extraction of [^{14}C]A across the splanchnic (hepatic) bed; and finally, (f) that portion of estrone that was derived by extraglandular aromatization of plasma androstenedione within the liver and splanchnic bed (as indicated by the difference in arterial-venous concentrations of [^{14}C]E1 in the hepatic vein and peripheral blood).

Study III was initiated by the continuous intravenous infusion of [6,7- ^3H]E1 and [4- ^{14}C]A in 5% dextrose and water containing 8% ethanol at 0.35 ml/min for 3 h through Teflon tubing utilizing a Harvard infusion pump (Harvard Apparatus Co., Inc., Millis, Mass.). 20 ml of blood was obtained before the infusion and at 2, 2½, and 3 h from the arm opposite the infusion site. After 2½ h of continuous tracer infusion, 20 ml of blood was also withdrawn from the hepatic vein through a percutaneous Teflon catheter placed with the aid of fluoroscopy. A 72-h urine sample was collected from the beginning of the infusion.

Study IV. Measurement of the transfer constant of conversion of plasma androstenedione to estrone sulfate, ($[\rho]_{\text{BB}}^{\text{AE1S}}$) and ($[\rho]_{\text{Bu}}^{\text{AE1S}}$); the production rate of estrone sulfate from plasma androstenedione (PPR-E1S); the daily production rate of estrone sulfate (PR-E1S); and the MCR-E1S. From the results of Studies I-III, it became apparent that the major product of extraglandular aromatization of plasma androstenedione that entered blood was not estrone but, likely, estrone sulfate. To confirm and to quantify this metabolic process, the tracers, [6,7- ^3H]E1S and [4- ^{14}C]A, were infused intravenously at a constant rate of 6 h; this study was performed when the subject was 11 yr 3 mo of age. Again, urine was collected for 72 h from the beginning of the infusion.

Tracer preparation and doses

The [4- ^{14}C]T was converted to [4- ^{14}C]A by oxidation with t-butyl chromate according to the method of Menini and Norymberski (18). Tracer purification methods have been described previously (16). The quantity of tracers infused were: Study I, [^3H]E1, 3.7 μCi + [^{14}C]A, 9.5 μCi ; Study II, [^3H]E2, 0.8 μCi + [^{14}C]T, 9.8 μCi ; Study III, [^3H]E1, 12.2 μCi + [^{14}C]A, 32.3 μCi ; and Study IV, [^3H]E1S, 81 μCi + [^{14}C]A, 18.4 μCi .

Processing of blood samples

All blood samples were collected into heparinized tubes and were processed as previously described (19). Briefly, after adding 2 mg of nonradioactive estrone sulfate, [^3H]A and [^3H]T as internal recovery standards, the steroids were extracted from plasma with 2 vol of methylene chloride. The residual plasma samples were treated with 1 ml methyl green per milliliter of plasma. This mixture was extracted twice with chloroform, and solvolysis was carried out utilizing the techniques described by Eberlein (20) and modified by Gant, et al. (21). Endogenous plasma androstenedione and testosterone concentrations were measured by a double-isotope derivative technique (22).

Urine collection and processing

All urine excreted by the patient was collected for 72 h after each tracer injection, and each 72-h urine pool was incubated with β -glucuronidase for 3 days at room temperature. The liberated steroids were extracted with ethyl acetate. Additionally, in Study IV, sulfurylated steroid conjugates were subjected to solvolysis after the extraction of steroids liberated by the glucuronidase treatment. Urinary estrone, estradiol, and estriol were isolated and purified from each extract by the techniques of gradient elution liquid-liquid Celite partition chromatography (Johns-Marville Perlite Corp., Joliet, Ill.), thin-layer chromatography, acetylation, and crystallization. The $^3\text{H}:\text{C}$ ratios were determined and the specific activities were measured as previously described (16).

RESULTS

Study I. The PR-E1 and the $[\rho]_{\text{Bu}}^{\text{AE1}}$. The results of Study I are presented in Table II. The computed transfer constant, $[\rho]_{\text{Bu}}^{\text{AE1}}$, was 0.44, and the PR-E1 computed from the specific activity of urinary [^3H]E1 (glucuronoside) was 317 $\mu\text{g}/\text{day}$. For comparison, the transfer constant, $[\rho]_{\text{Bu}}^{\text{AE1}}$, was measured in two normal prepubertal boys, ages 9 and 12 yr, and these data are also presented in Table II.

The differences among the $^3\text{H}:\text{C}$ ratios of urinary estrone, estradiol, and estriol are explicable in part by the results of subsequent studies; viz. infused [^{14}C]A was converted to [^{14}C]E1S, which suffers a metabolic fate different from that of the in vivo internal standard, [^3H]E1 (23). The specific activities of urinary [^{14}C]E1 and [^{14}C]E2 were similar. From this finding, we conclude that there was little or no estradiol formed independently of estrone sources. The finding that the specific activity of urinary [^3H]E3 was considerably less than the urinary [^3H]E1, may represent the direct

TABLE II

A Comparison of Transfer Constants of Conversion of Androstenedione to Estrone and Total Production Rates of Estrone in a Boy with Prepubertal Feminization and in Two Normal Prepubertal Boys

Study	Tracer dose	3H:14C ratio			Transfer constant, [ρ]AE1* BU	Specific activities				PRE1† μg/24 h	
		Urinary metabolites				E1	E2	E3			
									dpm 3H/μg		
I	0.39	G§	0.88	1.06	0.61	0.44	8,700	10,900	4,200	317	
III	0.38	G	0.79	0.85	0.64	0.48	—	—	—	—	
		S¶	0.70	0.90	—	(0.54)	12,000	16,500	—	(738)	
Normal male, 9 yr old	0.44¶	G	33.8	—	33.5	0.01	85,200	—	—	24	
Normal male, 12 yr old	0.38**	G	40.0	38.9	43.2	0.01	—	—	—	—	

* Transfer constant of the conversion of androstenedione to estrone measured from $^3\text{H}:^{14}\text{C}$ ratio of urinary estrone.

† Total production rate of estrone.

§ Estrogen glucuronosides.

¶ Estrogen sulfates.

¶ $[^3\text{H}]E1$ 2.8 μCi + $[^{14}\text{C}]A$ 6.4 μCi = tracer dose.

** $[^3\text{H}]E1$ 3.2 μCi + $[^{14}\text{C}]A$ 8.5 μCi = tracer dose.

formation of estriol from C_{19} prehormones, e.g. 16α -hydroxy androstenedione.

From the results of Study I alone, we could not determine what portion of the total production of estrogen was accounted for by glandular secretion and (or) by extraglandular mechanisms. The magnitude of the $[\rho]_{BU}^{AE1}$ value observed in this study suggested that the latter might be the principal source, but such a computation cannot be made without knowing the amount of plasma androstenedione available for aromatization.

Study II. The PR-E2 and $[\rho]_{BU}^{TE2}$. The $^3\text{H}:^{14}\text{C}$ ratio of the injected tracers $[^3\text{H}]E2$ and $[^{14}\text{C}]T$, in Study II was 0.08, whereas those of urinary estrone, estradiol, and estriol (glucuronosides) were 0.22, 0.51, and 0.14, respectively. The production rate of estradiol computed from the specific activity of urinary $[^3\text{H}]E2$, was 155 $\mu\text{g}/\text{day}$. From the $^3\text{H}:^{14}\text{C}$ ratio of urinary estradiol, the computed transfer constant, $[\rho]_{BU}^{TE2}$, was 0.16. Again, the dissimilarity of the $^3\text{H}:^{14}\text{C}$ ratios of urinary estrone, estradiol, and estriol in this study are explicable on the basis that multiple pathways are possible for the conversion of plasma $[^{14}\text{C}]T$ to estradiol, estrone, and estriol, compared to the more limited pathways available for the *in vivo* internal standard, $[^3\text{H}]E2$.

As in Study I, the specific activity of urinary estriol (600 dpm $^3\text{H}/\mu\text{g}$) was much lower than for estradiol (3,800 dpm $^3\text{H}/\mu\text{g}$) or estrone (1,500 dpm $^3\text{H}/\mu\text{g}$). From these data, it is apparent that more than one pathway was available for the formation of the estriol,

and the alternate pathway(s) was independent of estrone-estradiol formation. The similar specific activities of $[^{14}\text{C}]E1$ and $[^{14}\text{C}]E2$ in Study I and the higher specific activity of $[^{14}\text{C}]E2$ than $[^{14}\text{C}]E1$ in Study II again suggest that estradiol formation in this boy arose primarily from the reduction of estrone or estrone sulfate which was derived from extraglandular aromatization of plasma androstenedione.

Study III. The MCR-A, the PPR-A, the $[\rho]_{BU}^{AE1}$, the transsplanchnic extraction and conversion of plasma androstenedione to estrone, and the conversion ratio of androstenedione to testosterone, $[k]_{BU}^{AT}$, during the infusion of $[^{14}\text{C}]A$ and $[^3\text{H}]E1$. The $^3\text{H}:^{14}\text{C}$ ratios of the urinary estrogens isolated in Study III are presented in Table II. The $[\rho]_{BU}^{AE1}$, 0.48 (computed from the $^3\text{H}:^{14}\text{C}$ ratio of urinary estrone [glucuronoside]), was similar to that measured in Study I and is subject to the same qualifications discussed in that study. The values in parentheses (Table II) were computed from the $^3\text{H}:^{14}\text{C}$ ratio of the sulfate conjugates; but inasmuch as $[^3\text{H}]E1$ rather than $[^3\text{H}]E1S$ was infused, these values are only estimates of these pathways.

The data utilized in the computation of the metabolic clearance rate of androstenedione are presented in Table III. The similarity of plasma concentrations of $[^{14}\text{C}]A$ disintegrations per minute/liter) in three peripheral venous plasma samples indicates that a steady state was obtained between the rate of infusion of tracers and their disappearance from blood. The MCR-A was 2,667 liters/day or 2,380 liters/day per m^2 body surface. The PPR-A computed from the

TABLE III
Concentrations and Isotopic Ratios of Radioactive Steroids
in Peripheral and Hepatic Vein Plasma during In-
travenous Infusion of [¹⁴C]A and [³H]E1

Site	Hours	Estrone ³ H: ¹⁴ C ratio		Estrone sulfate		Andro- stenedione	Testos- terone
		³ H	¹⁴ C	ratio	dpm × 10 ³ /liter		
PV*	2	13.8	75	116	0.65	184	17
PV	2½	12.7	110	171	0.64	219	22
PV	3	10.3	150	241	0.62	232	22
HV†	2½	0.9	92	165	0.56	8	8

* Peripheral venous plasma.

† Hepatic venous plasma.

data of this study and the plasma concentration of androstenedione (Table I) was 1,173 µg/day.

The hepatic extraction of androstenedione was 96% (computed from the plasma concentration of [¹⁴C]A (disintegrations per minute/liter in the hepatic vein blood and in peripheral vein blood of the arm opposite the infusion site). Comparable data are not available for normal children, but this value is similar to that reported for the hepatic extraction of plasma androstenedione in normal adults by Rivarola et al. (24).

Since the ³H:¹⁴C ratios of plasma estrone did not become constant by the 3rd h of tracer infusion nor approach the ratios found in urine, the $[\rho]_{BB}^{AE1}$, could not be computed. Based on this finding, we considered the possibility that the estrogenic product formed from [¹⁴C]A in the tissue sites of aromatization, was sulfurylated *in situ* and entered the blood as estrone sulfate. Therefore, the plasma residues were reextracted for the sulfate conjugates and the ³H:¹⁴C ratios of estrone sulfate measured in each sample. It is evident from the data presented in Table III that the ratios of plasma estrone sulfate were much lower than those of plasma estrone, suggesting that the estrone formed from the aromatization of plasma androstenedione was sulfurylated within the sites of aromatization before it entered blood.

The data of this study did not permit calculation of the extent of aromatization of androstenedione across the splanchnic bed; such computations are possible only when the product of aromatization of androstenedione entering blood is estrone, when using infused [³H]E1, rather than estrone sulfate. The concentration of [³H]E1S in hepatic venous blood was 16% less than that in peripheral venous blood after a 2½ h infusion. Assuming that the concentration of [¹⁴C]E1S in plasma entering the liver was the same as that observed in peripheral plasma, and assuming that 84% of that amount of [¹⁴C]E1S would appear in the hepatic vein, the net entry of new [¹⁴C]E1S into the

hepatic vein after a 2½ h of infusion was 22,000 dpm [¹⁴C]E1S/liter of plasma. Assuming further a liver plasma flow of 850 liters/24 h in this boy², then 18,700,000-dpm [¹⁴C]E1S/24 h was generated in the liver from the infused 558,982,000-dpm [¹⁴C]A/24 h. The transsplanchnic transfer constant of conversion of plasma androstenedione to estrone sulfate was 0.03. If these assumptions are valid, then <6% of the total transfer constant of conversion of plasma androstenedione to estrone sulfate, $[\rho]_{BB}^{AE1S}$, occurred in the liver and (or) splanchnic bed because in Study IV, described below, the $[\rho]_{BB}^{AE1S}$ was 0.51.

The product of $[\rho]_{BB}^{AE1}$ and PPR-A in this study gives a computed PR-E1_A of 563 µg/day. From these data alone, it cannot be determined unequivocally whether estrone arose by direct secretion and by extraglandular formation in this boy since the PR-E1 was not measured independently in this study.

Study IV. Measurement of the transfer constant of conversion of plasma androstenedione to estrone sulfate ($[\rho]_{BB}^{AE1S}$) and ($[\rho]_{BB}^{AE1S}$); the production rate of estrone sulfate computed from the specific activity of urinary [³H]E1S (PR-E1S_A); the plasma production rate of estrone sulfate derived from plasma androstenedione (PPR-E1S_A); and the MCR-E1S. The data from this study are presented in Table IV. From the mean plasma concentrations of [¹⁴C]A, the MCR-A was computed to be 2,900 liters/day, a higher value than that computed in Study III. The PPR-A was 1,624 µg/day. The increased production rate of androstenedione was due not only to a difference in the metabolic clearance of androstenedione, but also to an increase in the plasma concentrations of androstenedione presumably from increased adrenal secretion of androstenedione at the time of this study (Table I). The ³H:¹⁴C ratios of estrone in plasma were measured and found to be similar to those observed in the previous study. From the ³H:¹⁴C ratios of plasma estrone sulfate, the transfer constant, $[\rho]_{BB}^{AE1S}$, was computed to be 0.51. The product of the $[\rho]_{BB}^{AE1S}$ and the PPR-A gives the PPR-E1S_A, 782 µg/day; where PPR-E1S_A is the plasma production rate of estrone sulfate derived from the extraglandular aromatization of plasma androstenedione (corrected for the change in molecular weight of precursor and product and expressed on the basis of the molecular weight of estrone).

From the concentration of [³H]E1S in plasma (disintegrations per minute/liter) during the infusion, the MCR-E1S was computed to be 396 liters/day. Even when expressed in relationship to body surface area, 353 liters/day per m², this value is three times

² Hepatic plasma flow was estimated as 850 liters/day from the data on Bradley et al. (25) for normal adult males. Body surface area of this boy was 1.12 m².

TABLE IV

Concentrations and Isotopic Ratios of Radioactive Plasma Steroids during a 6-h Intravenous Infusion of [¹⁴C]A and [³H]EIS

Hours	Estrone ³ H: ¹⁴ C ratio	Estrone sulfate			Andro- stenedione	Testos- terone
		³ H	¹⁴ C	³ H: ¹⁴ C ratio		
		dpm × 10 ⁹ /liter			dpm ¹⁴ C × 10 ⁹ /liter	
4	13.4	1,781	191	9.3	68	8.3
5	11.0	1,862	216	8.6	57	7.5
5½	11.7	1,785	207	8.6	55	6.5
6	11.0	1,770	230	7.7	50	6.6

that reported for normal adult males (26, 27); similar data for children have not been reported.

The ³H:¹⁴C ratios and specific activities of urinary estrone, estradiol, and estriol from both glucuronoside and sulfate hydrolysates are presented in Table V. The higher ratios observed in the sulfate fractions may indicate that some plasma androstenedione which was converted to estrone in tissue sites escaped sulfurylation and entered the plasma as estrone. The transfer constant, $[\rho]_{BU}^{AEIS}$, was 0.54 and was similar to that calculated from plasma data, where $[\rho]_{BB}^{AEIS}$ was computed to be 0.51. From these results, we conclude that most of the estrone formed from plasma androstenedione entered the plasma from the tissue sites of aromatization as estrone sulfate. From the specific activities of both the glucuronoside and sulfate estrogen fractions, it is again apparent that precursors other than androstenedione contributed to estriol formation independent of the intermediacy of estrone or estrone sulfate.

DISCUSSION

The purpose of this study was to quantify and determine the source of the endogenous estrogen formation in a prepubertal boy who developed severe feminization. After excluding an adrenal or testicular

source of estrogen secretion, we turned our attention to the measurement of extraglandular aromatization of the plasma C₁₉ prehormones, androstenedione and testosterone.

It is now well established that extraglandular formation of estrogen from plasma C₁₉ precursors contributes significantly to total estrogen production in both health and disease. Indeed, in normal postmenopausal women and in children, extraglandular aromatization of plasma androstenedione accounts for nearly all estrogen production. In young adult men, estrone formation occurs mainly by two mechanisms: (a) directly by the extraglandular aromatization of androstenedione to yield estrone and (b) indirectly by the conversion of plasma testosterone to estradiol which, in turn, is metabolized largely through estrone. Estradiol formation in young adult men arises principally by extraglandular formation from both plasma androstenedione and testosterone; however, small amounts of estradiol are secreted by the testes (28-30).

The advanced stage of feminization observed in this prepubertal boy was found to be the result of excessive estrogen formation from extensive aromatization of plasma androstenedione in extraglandular sites. However, the metabolic aberration responsible for the development of the heterosexual precocity was not limited to massive aromatization of plasma C₁₉ prehormones, but also involved extensive sulfurylation of estrone in the tissue sites of aromatization before entry of the estrogen product into plasma. This metabolic abnormality resulted in the daily formation of more than 800 µg of estrone sulfate in this boy.

Extraglandular aromatization accounted for at least 55% of the total clearance of plasma androstenedione in this boy. The transfer constant of conversion of androstenedione to estrone sulfate in this subject was more than 50 times that found in two normal prepubertal boys of similar age, and 30-40 times that found in normal young adult men (15).

Moreover, the extent of total aromatization of plasma androstenedione was probably even greater in that there was evidence that estriol was formed from plasma androstenedione by pathways other than those involving estrone or estrone sulfate; viz. there was direct evidence that estriol was formed by the pathway, androstenedione → 16α-OH-androstenedione → estriol. The specific activity of urinary [³H]E3 was lower than that of urinary [³H]E1 when either [³H]E1 or [³H]E1S was infused. These findings led to the conclusion that 16α-hydroxylation of plasma androstenedione was occurring, in part, before its aromatization to estriol. However, this source alone, i.e. 16α-hydroxy androstenedione, could not account totally for the decreased specific activity of estriol because the specific activity of urinary [¹⁴C]E3 was also less

TABLE V
Comparison of ³H:¹⁴C Ratios and Specific Activities of the Urinary Estrogens*

	³ H: ¹⁴ C ratios		Specific activities	
	Glucuronosides	Sulfates	Glucuronosides	Sulfates
<i>dpm ³H/µg</i>				
Estrone	5.8	7.9	80,000	67,600
Estradiol	6.2	7.7	74,500	76,900
Estriol	6.3	6.7	49,600	46,000

* Data from Study IV in which [³H]E1S and [¹⁴C]A were infused.

than that of [¹⁴C]E1 when [¹⁴C]A was infused. Other plasma precursors, e.g. 16 α -hydroxy dehydroisoandrosterone or its sulfate ester, served as C₁₉ prehormones for extraglandular estrone-independent formation of estriol. Thus, each contributing source of estriol that did not pass through estrone would serve to lower the specific activity of [³H]E3 compared to [³H]E1. From other studies reported from this laboratory, e.g. those of estrogen formation in pregnancy, it is known that 16 α -hydroxylation of C₁₉ precursors can occur before aromatization and result in estrone-independent estriol formation (31). Moreover, in such hyperestrogenic states as pregnancy, the extent of 16 α -hydroxylation of plasma C₁₉ steroids is strikingly increased (32).

Previously, the highest transfer constant of conversion of plasma androstenedione to estrone observed in our laboratory was 0.16, a value found in an obese, elderly postmenopausal woman with cirrhosis of the liver. The transfer constant of conversion of androstenedione to estrone may increase in obesity, hepatic disease, hyperthyroidism, and aging, but not to the extent observed in this subject (cf. 28).

The transfer constant of conversion of plasma testosterone to estradiol in this boy was 0.16, a value that is also approximately 50 times that found in normal young adult men (cf. 28). No data are available concerning the extent of conversion of plasma testosterone to estrogens in prepubertal children.

In this feminized prepubertal boy, the plasma production rate of estrone sulfate which was formed from plasma androstenedione, i.e. PPR-E1S_A = PPR-A \times $[\rho]_{BB}^{AE1S}$, was 782 μ g/day. This value compares to the PR-E1S_A, i.e. PR-E1S_A = PPR-A \times $[\rho]_{BU}^{AE1S}$, 828 μ g/day and to the PR-E1S, 879 μ g/day, which was computed independently from the specific activity of urinary [³H]E1S. These observations provide support for the conclusion that the massive extraglandular aromatization of plasma androstenedione in this boy resulted in the formation of estrone which, in turn, was sulfurylated in the tissue sites of aromatization before the estrogen product entered plasma.

At the time these studies were performed, the plasma concentration of estrone sulfate was not measured in this boy but was computed from the MCR-E1S and the PPR-E1S_A and estimated to be 1,975 pg/ml. Later, the plasma concentration of estrone sulfate was measured in this child and found to be 2,400 pg/ml. Ruder et al. (27), studying the kinetics of metabolism of estrone sulfate in normal young adult men, found a mean plasma production rate of estrone sulfate of 77 μ g/24 h and a plasma concentration of 460 pg/ml.

The plasma concentrations of testosterone found in this boy were within the range of values reported for normal prepubertal boys by Gandy and Peterson (33),

Saez and Bertrand (34), Rosenfield, et al. (35), and August, et al. (12). From the ratio of the concentrations of [¹⁴C]A to [¹⁴C]T in plasma in Study III during the intravenous infusion of [¹⁴C]A, the conversion ratio of plasma androstenedione to plasma testosterone $[k]_{BB}^{AT}$, was 0.096, a value similar to that reported by Horton and Tait (17). In Study IV, this conversion ratio of $[k]_{BB}^{AT}$ was 0.127. The product of this conversion ratio and the concentration of plasma androstenedione in Study III more than accounted for the plasma testosterone measured but, in Study IV, it did not account for all the plasma testosterone and may indicate that early testicular secretion of testosterone was occurring at the time of this study. Although no increase in testicular size was observed at this time, there had been a slow but progressive growth of axillary and pubic hair.

The aromatase enzyme activity must have been widely distributed in many tissues in this boy. The liver has been considered a probable site of aromatization in the fetus. However, in this boy, <6% of the total aromatization of plasma androstenedione was accounted for by hepatic and splanchnic metabolism. Moreover, the metabolic clearance of androstenedione (2,380 liters of plasma/m² per 24 h) was markedly increased in this boy compared to 970 liters/m² per 24 h usually cleared in children and adults (36). This high clearance of plasma androstenedione was the result of an increased extrahepatic, extraglandular aromatization, because more than 1,500 liters of plasma were cleared of androstenedione by extrahepatic aromatization.

In view of these findings and the observations that extensive aromatization (5-9) and sulfurylation (10) of steroids occur normally in fetal tissues, we hypothesize that the heterosexual procoity in this boy may have resulted from a failure of normal decrease in the expression of both the aromatase and sulfokinase enzyme activities after birth and that this metabolic abnormality led to the formation of massive amounts of estrone sulfate in extraglandular sites.

The signs of hyperestrogenism in this child were first noticed when he was 8 yr of age. In view of the nature of the metabolic abnormality that was responsible for the advanced feminization in this boy, the question arises as to why gynecomastia did not develop before he was 8 yr old. This particular age has been associated with a proposed maturational milestone referred to as adrenarche. If, indeed, adrenal maturation does begin at this age, then conceivably only then would enough plasma prehormone become available for sufficient extraglandular estrogen formation that could cause feminization. Several investigators have reported that the rate of urinary 17-ketosteroid excretion begins to rise at 7-8 yr of age (37). Saez and Bertrand (34) and Gandy and Peterson

(33) reported that a sharp rise in the plasma levels of dehydroisoandrosterone sulfate also began about 8 yr of age. Although these authors do not report striking changes in the plasma concentrations of androstanedione with age in these studies, they do report that the trend of the plasma concentration of androstanedione from ages 6-7 yr through puberty is slowly upward.

The production rates of androstanedione may increase in growing children without significant increases in the plasma concentration of androstanedione if the metabolic clearance rate increases in proportion to body growth. Horton and Frasier (36) found that the MCR-A in children with the adrenogenital syndrome was similar to that of normal adults when expressed as clearance per square meter of body surface area. Thus, even though the plasma concentration of androstanedione changes relatively little, the plasma production rate of androstanedione likely increases in children since the total plasma clearance increases as body surface area increases during the adolescent years. Using a mean MCR-A in children of 970 liters/m² per 24 h (36) and the 50th percentile for height and weight, the MCR-A at ages 2, 4, 6, 8, and 10 yr for boys would be 534, 679, 854, 922, and 1,020 liters/m² per 24 h, respectively. Using these computed clearance rates for androstanedione and the mean plasma concentrations of androstanedione in children of different ages (33), the production rates of androstanedione would be expected to be 280, 360, 450, 600, and 1,000 μ g/24 h, respectively, for each of these age groups of boys.

The MCR-A values of 2,667 and 2,900 liters/day observed in this subject exceed those values reported by Horton and Tait (17), Horton and Frasier (36), Gordon et al. (38), and Judd et al. (39) for normal adult men and women. The plasma levels of androstanedione in this boy were within the range reported by Gandy and Peterson (33) and by Frasier (40) for Stages I-II (Tanner) pubescent children. The product of the MCR-A and plasma concentrations of androstanedione gave PPR-A of 1,173 and 1,624 μ g/day in two studies. The values are lower than those reported for adult men and women or children with congenital adrenal hyperplasia. Thus, the hyperestrogenism observed in this boy probably did not occur until sufficient substrate, namely plasma androstanedione, became available at adrenarche. In addition, the *in situ* sulfurylation of the estrone formed in extraglandular sites of aromatization may have protected him, temporarily, against feminization.

The metabolic clearance rates of both plasma androstanedione and estrone sulfate were increased in this boy. The high MCR-A could be accounted for by the nature of the basic metabolic defect, i.e. massive extrahepatic extraglandular aromatization. The

marked increase in the metabolic clearance of estrone sulfate may be due, in part, to a more rapid urinary excretion of estrone sulfate. The rate of urinary excretion was observed to be increased where other sulfurylated steroid productions were increased, e.g. dehydroisoandrosterone sulfate (41).

From these studies, it cannot be ascertained which estrogen(s) caused the feminization. While most of the product hormone of aromatization, estrone, was sulfurylated in the tissue sites of aromatization before the entry of the estrogen into blood, we do not propose that feminization necessarily occurred through the action of estrone sulfate. Indeed, extensive hydrolysis of plasma estrone sulfate occurred in this child as evidenced by the plasma level of estrone and the recovery of large amounts of tritium-labeled estrogen glucuronosides after the infusion of [³H]E1S. Thus, feminization may have occurred through the action of estrone. Alternatively, plasma estradiol formed from estrone or even estradiol produced from estrone within the tissue sites of estrogen action may have been responsible for the expression of estrogen action.

In summary, we conclude that the advanced prepubertal feminization observed in the boy of this study resulted from massive extraglandular aromatization of plasma androstanedione and represents a heretofore undescribed metabolic aberration. The excessive estrogen production became clinically evident at 8 yr of age, perhaps at the time of the developmental milestone, adrenarche, a time when plasma androstanedione availability begins to increase in the prepubertal years.

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