Source of Estrogen Production in Postmenopausal Women[1]

J. M. GRODIN,[2] P. K. SIITERI, AND P. C. MACDONALD

Departments of Obstetrics, Gynecology and Biochemistry, The University of Texas Southwestern Medical School at Dallas, Dallas, Texas 75235

ABSTRACT. The purpose of the present study was to investigate the mechanisms by which estrogen production arises in the human female following the menopause. The possibilities considered were a) glandular secretion of estrone (E1) or estradiol (E2), b) the peripheral aromatization of circulating androstenedione (A), or c) the aromatization of other plasma precursors of estrone or estradiol.

Studies were carried out in 6 postmenopausal women in which the following were measured: the plasma production rate of A (PR^pA), the fraction of plasma androstenedione which was converted to estrone as measured in urine, $[\rho]^{AE1}_{BU}$, and in blood, $[\rho]^{AE1}_{BB}$, and the total estrone production rate as measured by the urinary method (PRE1). The mean PR^pA, 1.75 mg/24 hr, was about one-half of that observed in premenopausal women and arose principally from the adrenal glands. The mean transfer constant, $[\rho]^{AE1}_{BU}$ value was 0.027, which is about twice the value obtained in premenopausal women, suggesting an increase in conversion of A to E1 with age.

The results suggests that the principal estrogen formed in postmenopausal women is estrone and that it is derived by aromatization of plasma A. Further, essentially all estrone production in these women was accounted for by this process, since the mean quantity of E1 derived from A, $PRE1_A$, 45.4 μg/24 hr was virtually the same as the mean PRE1, 46.3 μg/24 hr. It is therefore likely that neither adrenal nor ovarian secretion of estrone or estradiol contributes significantly to total estrogen production in postmenopausal women. (*J Clin Endocrinol Metab* **36**: 207, 1973)

PREVIOUS STUDIES in this laboratory have shown that of all the potential androgen precursors of estrone[3] and/or estradiol normally present in human plasma, circulating androstenedione (A), is most efficiently aromatized in both males and nonpregnant females (1–3). Furthermore, it has been demonstrated that approximately 1.3% of androstenedione entering the circulation is converted to the product hormone, estrone, in the normal, in the castrate, and in the adrenalectomized young adult female (3). These findings suggested that estrogen production in the human arises not exclusively by glandular secretion but additionally by "extraglandular formation" via the utilization of circulating C_{19} steroidal precursors at sites other than the ovaries or the adrenal glands.

The postmenopausal woman provides an excellent subject for the extension of these studies of estrogen production from plasma precursors since it is well known that there is a marked decrease in the level of ovarian estrogen secretion following the cessation of ovulatory cycles (4). It was speculated that in the postmenopausal woman, estrone production resulting from aromatization of circulating androstenedione might account for a large proportion of total estrogen production. Estrone may be produced by a) glandular biosynthesis and secretion of estrone, b) aromatization of circulating androstenedione, c) formation of estrone from plasma precursors other than androstenedione, or d) formation of estrone via the metabolism of secreted or estrone-independently-produced estradiol. It follows, therefore, that if total estrone production can be accounted for by aromatization of plasma A, one must conclude that these other possible mechanisms of estrone production are not operative. Most importantly, one would conclude that

Received March 15, 1972.

[1] This study was supported in part by USPHS Grant AM-06912.

[2] Present Address: National Institutes of Health, Bethesda, Maryland.

[3] The following trivial names and abbreviations are used in the text: A = androstenedione = androst-4-ene-3,17-dione; T = testosterone; E1 = estrone; E2 = 17β-estradiol.

little, if any, estradiol was being produced either by secretion or by formation from other plasma precursors. This would be true since estradiol is metabolized principally via estrone (5).

As will be seen from the results of this study in postmenopausal subjects, the PRE1 and $PRE1_A$ were essentially equal. Accordingly, in these subjects, PRE1 represents an estimate of total estrogen production with respect to the estrone–estradiol system. The independent formation of estriol cannot be excluded on the same theoretical grounds but it was not detected by a comparison of the specific activities of urinary estrone and estriol.

Materials and Methods

From each subject studied, approximately 50 ml of heparinized blood was drawn every 6 hr during one 24-hr period. The plasma samples obtained from these collections were pooled and, after the addition of internal standards, were stored at —20 C prior to measurement of the concentration of androstenedione. On the following day each subject received a constant intravenous infusion of 6,7-^{3}H-estrone (11.6–15.6 μCi) contained in 0.5–1.0 liter of 5% dextrose–5% ethanol in water. The tracers were administered during approximately 4½ hr using a peristaltic pump fitted with Teflon tubing. Heparinized blood specimens (Ca 50 ml) were drawn after 3, 3½, and 4 hr of tracer infusion. Urine was collected for 72 hr from the beginning of the infusion.

Determination of the plasma production rate of androstenedione (PR^pA). The metabolic clearance rate of androstenedione (MCR-A) was calculated from the equilibrium concentration of 4-^{14}C-androstenedione in plasma (dpmA-^{14}C/L plasma) during the continuous intravenous infusion of 4-^{14}C-androstenedione (6). To each of the measured plasma samples, 95,000 dpm of 7α-^{3}H-androstenedione plus 200 μg each of nonradioactive androstenedione and estrone were added. The samples were then extracted 2 times with equal volumes of methylene chloride. The methylene chloride extracts were pooled and washed 2 times with water. The methylene chloride was evaporated to dryness, and this extract was purified by celite partition chromatography utilizing increasing quantities of ethyl acetate as the mobile phase as previously described (7).

TABLE 1. Thin-layer chromatography systems

Designation	Solvents	Volume ratio of solvents
A	Ether:methylene chloride	12:88
B	Iso-octane:ethyl acetate	60:40
C	t-butanol:benzene	3:97
D	Ether:methylene chloride	10:90
E	Ethanol:benzene	20:80

The eluate fractions containing androstenedione were located by assay of radioactivity in 10% aliquots, and the fractions containing estrone were located by applying small aliquots to Whatman filter paper, followed by staining with Folin's phenol reagent. The androstenedione eluate was then further purified by successive thin-layer chromatography (Systems B & A, Table 1). Thereafter, crystallization was repeated until mother liquors and crystals contained identical ^{3}H/^{14}C ratios. The concentration of 4-^{14}C-androstenedione in the plasma samples was calculated from the final ^{3}H/^{14}C and the amount of ^{3}H-androstenedione added to the plasma.

Utilizing the principle of exchanging enolic hydrogens for tritium described by Siiteri *et al.* (8), the plasma concentration of androstenedione was determined according to the method of Siiteri (7). Each subject's plasma pool was divided into 2 equal parts and to each approximately 2000 dpm of 4-^{14}C-androstenedione (SA ~ 400,000 dpm/μg) was added. An identical amount of tracer was added to a 20-ml water blank which was simultaneously processed in the same manner as the plasma. The plasma samples and water blanks were then adjusted to pH 10–10.5 and extracted twice with equal volumes of methylene chloride. The methylene chloride extracts of each sample were combined and washed 2 times with equal volumes of water. The organic extract was then evaporated and the residue partitioned between 70% methanol-water and heptane. The 70% methanol fraction was then diluted with sufficient water to reduce the methanol concentration to 25% and was ex-

tracted with methylene chloride. The methylene chloride extract was subsequently evaporated, dissolved in benzene-n-hexane 1:1 and applied to a 1.0 × 10 cm column containing 0.5 g Woelm basic alumina and 6% water. After washing the column with 10 ml of the same solvent mixture, androstenedione was eluted from the column with benzene-n-hexane containing 0.5% ethanol. The eluate was transferred to a screw-top glass culture tube and dried under a stream of nitrogen. The concentration of androstenedione was determined following labeling with AL_2O_3–^3H_2O as previously described (8).

Determination of the extent of conversion of plasma androstenedione to estrone as measured in urine, $[\rho]^{\Delta E1}_{BU}$, *and blood,* $[\rho]^{\Delta E1}_{BB}$. The total extent of conversion of circulating androstenedione to estrone, $[\rho]^{\Delta E1}_{BU}$, was determined from the isotope ratio of the infused tracers and that of estrone isolated from the glucuronide fraction of the 3-day urine collection, as previously described (3). The fraction of plasma androstenedione converted to free estrone in plasma, $[\rho]^{\Delta E1}_{BB}$, was similarly calculated using the isotope ratio of estrone isolated from plasma collected during the infusion.

The estrone fraction from the gradient elution celite column was subjected to successive thin-layer rechromatography (Systems D, B, & E, Table 1). Following purification in each system, 10% of the sample was assayed for radioactivity. Identical $^3H/^{14}C$ ratios following successive rechromatography were taken as evidence for radiochemical homogeneity, and the mean of the last 2 ratios was used in the calculation of $[\rho]^{\Delta E1}_{BB}$.

Production rate of estrone from androstenedione ($PRE1_A$). Knowing the amount of circulating androstenedione produced and the fraction which was converted to estrone, the total amount of estrone derived by this conversion was then calculated from the relationship: $PRE1_A = (PR^pA) \cdot [\rho]^{\Delta E1}_{BU}$. Similarly, the quantity of estrone ($PRE1^p_A$) derived from circulating androstenedione which actually entered the circulation as free hormone was calculated as follows: $PRE1^p_A = (PR^pA) \cdot [\rho]^{\Delta E1}_{BB}$. This amount represents a minimum estimate of estrogen physiologically available to target tissues.

Total estrone production rate (*PRE1*). The total production rate of estrone was calculated from the cumulative specific activity of urinary estrone glucuronide and the amount of ^3H-estrone administered during the infusion by the classical urinary method as previously described (9). This calculated amount represents the average *total* production rate of new estrone regardless of its mechanism of origin. The specific activities of estrone, estradiol, and estriol were determined by the isotope derivative technique after acetylation of separate samples of each metabolite with ^3H-labeled and nonradioactive acetic anhydride. The acetates thus obtained were purified by thin-layer chromatography and recrystallization with nonradioactive standards until the $^3H/^{14}C$ ratios of successive crystals and mother liquors were identical. The specific activities of estrone, estradiol, and estriol were then calculated as previously described (9).

Subjects

1. PH 51-yr-old Negro, Gr X, Para V, Abortus V, 4 years postmenopausal; asymptomatic.
2. LS 53-yr-old white, Gr V, Para V, Abortus O, 5 years postmenopausal; asymptomatic.
3. RB 56-yr-old white, Gr V, Para V, Abortus O, 3 years postmenopausal, admitted for the evaluation of stress incontinence and the treatment of uterine descensus with cystorectocoele.
4. LF 61-yr-old Negro, Gr II, Para I, Abortus I; supracervical hysterectomy 1942 for "abdominal mass"; 1964—removal of 10 × 20 cm cystic mass "arising from cul-de-sac"—pathological diagnosis: serous cystadenocarcinoma. No other ovarian tissue found. Four years postoperative, patient asymptomatic.
5. RR 52-yr-old white, Gr II, Para I, Abortus I; bilateral salpingectomy 1946 for "infection"; right radical mastectomy and subsequent irradiation 1957 for carcinoma of the breast; menses extremely light and irregular for 3 years prior to November 1966, when she had bilateral oophorectomy due to right pleural effusion with

cytology showing adenocarcinoma. For the past 1½ yr, the patient has had no evidence of recurrence.

6. ES 51-yr-old Indian, Gr VI, Para V, Abortus I; carcinoma of the cervix, Stage I; adult onset diabetes. Admitted for radium implant. Menopause occurred 9 yr prior to study.

Results

The $^{3}H/^{14}C$ ratios of the estrogen metabolites isolated from urine following the simultaneous intravenous infusion of 4-^{14}C-androstenedione plus 6,7-^{3}H-estrone and the calculated rho values are shown in Table 2. As evidenced by the near identity of the $^{3}H/^{14}C$ ratios of urinary estrone, estradiol, and estriol in each experiment, it is apparent that the estrogen derived from administered 4-^{14}C-androstenedione behaved identically with the *in vivo* internal standard, 6,7-^{3}H-estrone. Accordingly, the product estrogenic hormone derived from plasma androstenedione in these subjects was estrone, as was previously observed in premenopausal patients (3). The total extent of conversion of circulating 4-^{14}C-androstenedione to 4-^{14}C-estrone, $[\rho]^{AE1}_{BU}$, in this group of subjects ranged from 1.6–3.6%, and the mean value was 2.7%. This value is about twice that observed in endocrinologically normal, adrenalectomized, and oophorectomized young adult women (3), or in normal men and women as estimated in blood by Longcope *et al.* (10).

TABLE 2. Percent conversion of androstenedione to estrone $[\rho]^{AE1}_{BU}$

Subject	$^{3}H/^{14}C$ ratio (dpm of urinary estrogens*) Estrone	Estradiol	Estriol	$[\rho]^{AE1}_{BU}$ (%)
PH	26.4	—	23.8	1.6
LS	13.0	13.5	13.9	3.2
RB	13.4	12.7	14.0	3.1
LF	11.7	11.3	12.8	3.6
RR	17.3	17.5	18.4	2.4
ES	18.5	17.8	21.5	2.3

* For ease of comparison, all $^{3}H/^{14}C$ ratios have been corrected to a common tracer dose of 30.0×10^{6} dpm of ^{3}H-estrone and 70.0×10^{6} dpm ^{14}C-androstenedione.
$^{3}H/^{14}C$ ratio of infused tracers = 0.428.

TABLE 3. Production rate of estrone from plasma androstenedione*

Subject	C_A (μg/l)	MCR-A (l/24 hr)	PRA^P (mg/24 hr)	$[\rho]^{AE1}_{BU}$ (%)	$PRE1_A$ (μg/24 hr)
PH	1.03	1592	1.64	1.6	24.7
LS	0.83	1717	1.43	3.2	43.1
RB	1.05	2065	2.17	3.1	63.5
LF	0.94	1926	1.81	3.6	61.5
RR	0.71	1154	0.82	2.4	18.6
ES	1.04	2548	2.65	2.3	61.2
Mean	0.93	1834	1.75	2.7	45.4

* The production rate of estrone derived from androstenedione is corrected for the difference in the molecular weight of androstenedione and estrone.

As seen in Table 3, the plasma concentrations of androstenedione in these postmenopausal women (mean, 0.93 μg/l) were somewhat lower than those previously reported in premenopausal women by other workers using different techniques (11). The mean MCR-A was 1834 l/24 hr and is not significantly different from values reported by other laboratories for normal females (11, 12). The plasma production rates of androstenedione, *i.e.*, the product of the metabolic clearance rates and the plasma concentration of androstenedione shown in Table 3, are somewhat less than those reported by Baird *et al.*, in normal premenopausal women (12). The average production rate of estrone from circulating androstenedione was 45.4 μg/day and ranged from 18.6–63.5 μg/day.

The specific activities of the urinary estrogens and the calculated total daily production rates of estrone for each subject are shown in Table 4. The total daily production rate of estrone ranged from 15.8–76.5 μg/24 hr, with an average of 46.3 μg/24 hr. In only

TABLE 4. Total daily estrone production rate Specific activity urinary estrogen (dpm $^{3}H/\mu g$)*

Subject	Estrone	Estradiol	Estriol	Production rate estrone (μg/24 hr)
PH	450,500	—	—	22.2
LS	264,600	261,782	291,616	37.8
RB	169,200	154,172	136,356	59.1
LF	130,700	119,755	73,771	76.5
RR	632,900	576,556	592,593	15.8
ES	150,300	130,200	158,400	66.5

* The specific activities have been normalized to a common tracer dose (^{3}H-estrone = 30.0×10^{6} dpm) and molecular weight (270) for ease of comparison.

one subject (LF) was there a significant difference in the specific activities of the 3 metabolites. The much lower specific activity of estriol as compared to estrone and estradiol suggests the possible estrone–estradiol independent formation of estriol in this subject.

The near identity of the $^{3}H/^{14}C$ ratios of estrone isolated from the 3- and 4-hr plasma samples obtained during the tracer infusion suggests that equilibrium between the rate of infusion of 6,7-^{3}H-estrone, the rate of formation of ^{14}C-estrone derived from infused 4-^{14}C-androstenedione, and their rates of disappearance from plasma had been achieved (Table 5). The amount of carbon-14 labeled estrone available in plasma for the determination of the $^{3}H/^{14}C$ was approximately 3000–6000 cpm/l plasma. From the $^{3}H/^{14}C$ ratio of the mixture of infused tracers and that of plasma estrone at equilibrium, the values of $[\rho]^{AE1}_{BB}$ and the percentage of total estrone derived from circulating androstenedione which actually entered the plasma were calculated and are shown in Table 5. As can also be seen in Table 5, the mean plasma production rate of estrone, $PRE1^{p}_{A}$, was 21.2 μg/24 hr.

Discussion

Until recently it was generally held that the human adrenal glands secrete significant amounts of estrogens even though little direct evidence for this belief had been provided. Numerous studies in postmenopausal and oophorectomized women demonstrated that such individuals continue to excrete measurable amounts of urinary estrogens. The adrenal glands were implicated as the source of these estrogens since the administration of ACTH caused an increase, whereas adrenalectomy led to a decrease in the levels of urinary estrogens. For example, Brown *et al.* (13), found that total urinary estrogen excretion by an oophorectomized woman exceeded that observed for ovulatory women after 2 days' treatment with ACTH. Also, some direct evidence for adrenal secretion of estrogens was recently reported by Baird *et al.*, who demonstrated higher estrone levels in adrenal venous blood than in peripheral blood following ACTH administration in 2 of 3 subjects (14). However, since no differences were noted under basal conditions, the significance of these findings is presently unclear.

Several observations suggested an alternative mechanism of estrogen production in the postmenopausal woman. The conversion of parenterally administered radioactive androgens to urinary estrogen in nonpregnant women has been demonstrated by several workers (reviewed in Ref. 3). These qualitative findings, together with the demonstration of the quantitative importance of adrenal "androgen" precursors for placental

TABLE 5. Entry into blood of estrone derived from plasma androstenedione

Subject	$^{3}H/^{14}C$ Ratio (dpm) Plasma* estrone	$^{3}H/^{14}C$ Ratio (dpm) Urinary estrone	$[\rho]^{AE1}_{BB}$ (%)	% of Estrone derived from plasma androstenedione which entered plasma	$PRE1^{p}_{A}$ (μg/24 hr)
PH	34 (31, 37)	26.4	1.2	78	19.3
LS	29.5 (28, 31)	13.0	1.4	44	19.0
RB	46 (47, 45)	13.4	0.9	29	18.4
LF	21.5 (21, 22)	11.7	1.9	54	33.2
RR	22.5 (24, 21)	17.3	1.8	77	14.3
ES	46.5 (49, 44)	17.2	0.9	37	23.0
Mean			1.35	53	21.2

* The average $^{3}H/^{14}C$ ratios of plasma estrone are shown together with the final observed values obtained after multiple purification steps (see text) from the 3- and 4-hr plasma samples indicated in parentheses.

estrogen production during pregnancy (1), led to the hypothesis that extraglandular estrogen production in the human may arise from C_{19} steroidal precursors which are normally present in the peripheral circulation (3). The present data confirm our earlier report which demonstrated the quantitative importance of this process (15).

The blood production rates of androstenedione (mean = 1.75 μg/24 hr) in these postmenopausal women are about one-half of those reported for premenopausal women in the follicular phase of the menstrual cycle (16). Although the lowest value (0.82 μg/24 hr) was obtained in 1 of the oophorectomized subjects (RR), it would appear that the adrenal glands were the principal source of androstenedione in these subjects. Variations in the production rate due to diurnal changes in secretion and plasma levels were minimized by pooling plasma samples taken at 6-hour intervals over 24 hr. Thus, the androstenedione blood production rate and the calculated estrone production rate derived from androstenedione represent mean values. This may account in part for the relatively good agreement between total estrone production and the contribution to this total made by peripheral conversion of plasma androstenedione to estrone.

The average extent of conversion of plasma androstenedione to estrone in these postmenopausal women was 2.7% as compared to an average value of 1.3% in premenopausal women which we previously reported (3). An increase in conversion of circulating precursors to estrogen (androstenedione → estrone and testosterone → estradiol) with advancing age has been observed in male subjects (17), as well as in certain postmenopausal women who have evidence of excessive estrogen production (unpublished results). In this connection it is of interest that other workers have reported a steady rise in urinary estrogen excretion following the menopause (18,19). The present findings could provide a plausible explanation for this paradoxical phenomenon. The average production rate of estrone arising from this conversion was 45.4 μg/24 hr, and, as was expected, this value is considerably lower than the values found at midcycle in premenopausal women by Goering and Herrmann (20). Also, as one would expect, our values are considerably lower than the urinary estradiol production rates measured in postmenopausal women treated with ACTH, as reported by Barlow *et al.* (21). However, the recent report by Longcope (22) in which the blood production rate of estrone in postmenopausal woman estimated from mean values of MCR and plasma level was calculated to be about 40 μg/24 hr is in agreement with our findings. Earlier estimates of blood production rates of estrone in both men and women were much greater, due presumably to overestimation of both endogenous levels and the metabolic clearance rate (16).

Of more importance, however, is the striking agreement between the production rate of estrone derived from androstenedione and the total estrone production rate (45.4 *vs.* 46.3 μg/24 hr, respectively). Thus, within the limits of technical error which can be estimated to be around 15% in each experiment, it must be concluded that the major source of estrogen in the postmenopausal female is peripheral formation of estrone from plasma androstenedione and not ovarian or adrenal secretion; that the results obtained in the absence of ovaries, subjects LF and RR, were essentially the same as those obtained in the other 4 subjects strengthens this conclusion. Longcope recently has reached a similar conclusion in apparently normal postmenopausal women using quite a different experimental approach (22). These results have obvious implications concerning the use of ablation therapy for various endocrinopathies and neoplastic disease.

Although production of estrone from circulating androstenedione accounted for most of the estrogen production in these women,

the actual amount of estrone entering plasma was substantially less than the total produced in each case. The reason for the differences in the percentage of $PRE1_A$ which entered the circulation in these subjects (Table 5) is not immediately apparent. It is interesting, however, to compare both the $[\rho]^{\Delta E1}_{BU}$ values and the percentage of total estrone derived from androstenedione that enters the circulation with the body weight of each subject. While these limited data do not permit firm conclusions, when taken together with results obtained in a large series of studies in postmenopausal women with uterine bleeding they suggest that there may be a positive correlation between the extent of conversion of androstenedione to estrone and body weight. On the other hand, there appears to be a negative correlation between body weight and the percentage of estrone derived from plasma androstenedione which enters the circulation. These findings tend to implicate adipose tissue as a significant site of production and/or metabolism of estrone derived from circulating androstenedione. This would explain the higher conversions observed in the more obese subjects who were otherwise of similar size and age. Additionally, the inverse correlation of percentage of estrone entering plasma and body weight might be explained by slow release of estrone derived from androstenedione from body fat depots. Unpublished experiments have demonstrated the conversion of androstenedione to estrone, albeit in very low yields, during *in vitro* incubation of defatted human adipose tissue.

It should be pointed out that despite the fact that estrone isolated from the 3- and 4-hr plasma samples had similar isotope ratios in each subject, it is possible that equilibrium had not been reached between the rate of infusion and disappearance of 6,7-^{3}H-estrone and that of entry of ^{14}C-estrone into plasma due to a slow rate of conversion of androstenedione to estrone. Thus, the ultimate extent of entry into the circulation of androstenedione-derived estrone may have exceeded that calculated in these studies. An analogous situation has recently been reported by Longcope and Tait, who found that the concentration of estrone in plasma continues to rise up to 12 hr during the infusion of ^{3}H-estradiol (23). On the other hand, it is quite possible that estrone formed from androstenedione was released from the tissue site of aromatization both as free estrone and estrone sulfate. Twombley and Levitz demonstrated that estrone sulfate gives rise to urinary metabolites conjugated with glucuronic acid, much the same as does free estrone, indicating similar metabolic patterns (24). More recently, it has been shown that estrone sulfate, while giving rise to free estrone in blood, has a much lower metabolic clearance rate in both males and females than does estrone (25,26). Taken together, these observations would account for the present finding that total estrone production can be accounted for by the conversion of plasma androstenedione, even though the $[\rho]^{\Delta E1}_{BB}$, measured after 3–4 hr of infusion, is considerably less than $[\rho]^{\Delta E1}_{BU}$. Preliminary studies to test this possibility, *i.e.*, the formation of estrone sulfate directly from androstenedione, appear promising.

The results of these studies suggest that increased estrogen production in the postmenopausal woman may occur as the consequence of an increased conversion of plasma androstenedione to estrone or, alternatively, via an increased availability of blood androstenedione. The former situation has been observed in a number of postmenopausal women, resulting in increased estrone production, endometrial hyperplasia, and postmenopausal uterine bleeding. The second alternative has also been observed in several postmenopausal women with ovarian tumors. Furthermore, in none of these studies has evidence been obtained for adrenal or ovarian secretion of estrone or estradiol in postmenopausal women. The results of these studies are to be reported elsewhere.

Acknowledgment

The authors gratefully acknowledge the expert technical assistance of Mr. Frank Hereford and Miss Kay Cox.

References

1. Siiteri, P. K., and P. C. MacDonald, *Steroids* **2**: 713, 1963.
2. MacDonald, P. C., and P. K. Siiteri, *Excerpta Medica Int Cong Series* **111**: 151 (Milan), 1966 (Abstract).
3. MacDonald, P. C., R. P. Rombaut, and P. K. Siiteri, *J Clin Endocrinol Metab* **27**: 1103, 1967.
4. Procope, B., *Acta Endocr Suppl* **60**: 135, 1969.
5. Fishman, J., J. L. Bradlow, and T. F. Gallagher, *J Biol Chem* **235**: 3104, 1960.
6. Tait, J. F., and S. Burstein, *In* Pincus, G., K. V. Thimann, and E. B. Astwood (eds.), The Hormones, **5**: 441, New York, Academic Press, 1964.
7. Siiteri, P. K., *Excerpta Medica Int Cong Series* **184**: 476, 1969.
8. Siiteri, P. K., C. Tippet, C. Yates, and J. C. Porter, *Endocrinology* **82**: 837, 1968.
9. Siiteri, P. K., *Steroids* **2**: 687, 1963.
10. Longcope, C., K. Tatsuo, and R. Horton, *J Clin Invest* **48**: 2191, 1969.
11. Horton, R., and J. F. Tait, *J Clin Invest* **45**: 301, 1966.
12. Baird, D., R. Horton, C. Longcope, and J. F. Tait, *Perspect Biol Med* **11**: 384, 1968.
13. Brown, J. B., C. W. A. Falconer, and J. A. Strong, *J Endocrinol* **19**: 52, 1959.
14. Baird, D. T., A. Uno, and J. C. Melby, *J Endocrinol* **45**: 135, 1969.
15. MacDonald, P. C., J. M. Grodin, and P. K. Siiteri, *Excerpta Medica Int Cong Series* **184**: 770, 1968.
16. Baird, D. T., R. Horton, C. Longcope, and J. F. Tait, *Recent Progr Horm Res* **25**: 611, 1969.
17. MacDonald, P. C., J. M. Grodin, and P. K. Siiteri, *In* Baird, D. T., and J. A. Strong (eds.), Control of Gonadal Steroid Secretion, Edinburgh University Press, Edinburgh, 1971, p. 158.
18. Pincus, G., L. P. Romanoff, and J. Carlo, *J Gerontol* **9**: 113, 1954.
19. Nissen-Mayer, R., *Clin Radiol* **15**: 152, 1964.
20. Goering, R. W., and W. L. Herrmann, *J Clin Endocr* **26**: 65, 1966.
21. Barlow, J., K. Emerson, and B. N. Saxena, *N Eng J Med* **280**: 633, 1969.
22. Longcope, C., *Am J Obstet Gynecol* **111**: 778, 1971.
23. Longcope, C., and J. F. Tait, *J Clin Endocrinol Metab* **32**: 481, 1971.
24. Twombley, G. H., and M. Levitz, *Am J Obstet Gynecol* **80**: 889, 1960.
25. Ruder, H. J., D. L. Loriaux, and M. B. Lipsett, *The Endocrine Society Program of the 53rd Meeting,* San Francisco, California, 1971, A-141 (Abstract).
26. Longcope, C., *J Clin Endocrinol Metab* **34**: 113, 1972.