

Serum Estrogen and Androgen Levels following Treatment for Cervical Cancer

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Abstract

Endogenous sex hormones seem to influence the risk of several common and debilitating diseases. With a view toward better understanding the effects of surgical removal of the ovaries and high-dose pelvic radiotherapy on plasma sex hormone levels, we measured estrogen and androgen concentrations cross-sectionally among 147 women who had been treated for cervical cancer 0.3–18.5 years previously. Pelvic radiotherapy (mean dose to ovaries, 50 Gy) and bilateral ovariectomy were associated with similarly reduced hormone concentrations relative to levels among nonirradiated women with intact ovaries, most of whom had had early-stage disease and were treated by hysterectomy. There was little evidence that radiotherapy in addition to ovariectomy further lowered concentrations below levels associated with ovariectomy alone, such as might be expected if radiation was suppressing adrenal endocrine function. Among women age 50 years or older at the time of blood drawing, the removal or irradiation of the ovaries was associated with approximately 45% lower concentrations of estradiol (mean ratio [MR], 0.55; 95% confidence interval [CI], 0.32–0.95) and testosterone (MR, 0.57; 95% CI, 0.32–0.99), and 25–30% lower concentrations of estrone (MR, 0.69; 95% CI, 0.44–1.09) and androstenedione (MR, 0.76; 95% CI, 0.47–1.23), relative to the hysterectomy-only group. Among women younger than 50, ovariectomy and radiotherapy, alone or in combination, were associated with 83% lower estradiol concentrations (MR, 0.17; 95% CI, 0.09–0.31), 46% lower estrone concentrations (MR, 0.54; 95% CI, 0.37–0.81), 23% lower androstenedione concentrations

(MR, 0.77; 95% CI, 0.57–1.04), and 14% lower testosterone levels (MR, 0.86; 95% CI, 0.64–1.15). A possible mechanism for the reductions among postmenopausal women involves effects on androgen-producing cells in the ovary, which retain secretory function after menopause.

Introduction

Levels of steroid sex hormones are associated with the risk and natural history of a variety of diseases, including cancers of the breast and endometrium, cardiovascular disease, and osteoporosis (1–8). Physiological or “natural” menopause and bilateral ovariectomy or high-dose ovarian irradiation among premenopausal women are all associated with substantial changes in levels of endogenous reproductive hormones, yet the three events do not have identical implications for overall sex hormone profiles and might, by extension, differentially influence the risk of hormone-dependent diseases. Postmenopausal ovaries continue to secrete androgens (9–11), some of which are converted peripherally to estrogens (12, 13). Bilateral ovariectomy eliminates all ovarian sources of hormones. With irradiation, there is the possibility of gradations of tissue damage and impairment of secretory function, depending on radiation type, dose, dose-rate, age at exposure, and other factors, not only to cells in the ovary but to other, nearby endocrine tissues as well.

The observations that pelvic radiotherapy for cervical cancer (14) and benign gynecological disorders (15) appeared to be associated with a reduced risk of breast cancer, even among women irradiated postmenopausally, indicated that irradiation might have a protective effect apart from that associated with inducing an early menopause. Among long-term (~20 y²) survivors of cervical cancer whose ovaries had been removed, plasma levels of androstenedione, testosterone and estrone were lower among women treated with radiation than among nonirradiated patients (16), although significantly so only for androstenedione. These findings raised the possibility that irradiation might influence circulating androgen and estrogen levels through effects on extraovarian sites, such as the adrenal glands. To better understand how serum androgen and estrogen levels are influenced by irradiation and ovariectomy, we measured plasma concentrations for a second sample of cervical cancer patients.

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² The abbreviations used are: BMI, body mass index (relative weight); CI, confidence interval; CV, coefficient of variation; Gy, gray; MR, mean ratio; $P_{(1)}$, one-sided *P*-value; $P_{(2)}$, two-sided *P*-value; y, year.

Table 1 Distribution of the 147 cervical patients by treatment category and age at time of blood draw.

	Radiotherapy			
	<50 y		≥50 y	
Bilateral Ovariectomy	no	yes	no	yes
no	28	16	4	63
yes	2	7	19	8

Materials and Methods

Study Population. Eligible women were diagnosed with invasive or *in situ* cancer of the uterine cervix between 1966 and 1985 at one of three hospitals in Boston, Massachusetts, Buffalo, New York, or Houston, Texas, treated by irradiation and/or surgery, still living in 1985, and resident in the vicinity of the hospital. A minimum of 4 months had passed since first treatment for cervical cancer. Women with a history of liver disease or chemotherapy and those who reported having used exogenous estrogens within the 2 weeks prior to phlebotomy were excluded. A sample of nine recently diagnosed (as of 1985) women also was identified, to permit the collection of pre- and post-treatment (4–12 months) samples for the same woman. Analysis is based on hormone measurements for 147 women, 53 of whom were younger than 50 y at the time of phlebotomy and 94 of whom were ≥50 y (Table 1). Subject enrollment did not account for ovarian status or age at time of blood draw because the study population was assembled, in part, for a study of chromosome aberrations in relation to radiation exposure. For this reason, sample size and age distribution are not balanced with respect to the radiotherapy and ovariectomy treatment categories used in this analysis. Only four samples were obtained for women age 50 y or greater at the time of phlebotomy who had not been irradiated and who still had intact ovaries.

Blood Collection and Hormone Measurements. Letters were sent to potentially eligible women, asking them to participate in the study. For those who consented and were found to be eligible upon completion of a brief screening questionnaire, blood samples (30 ml) were collected from 1985 to 1986 at the participating clinic (usually) or the subject's home and shipped by overnight courier, with a freezer pack, to a commercial laboratory. Samples were collected in the morning, between 8:00 and 10:00 a.m., whenever possible, and in any case the time was recorded. Sera were stored at –20° until early 1991, when they were prepared for hormone measurements.

Levels of bound plus unbound estradiol, estrone, androstenedione, and testosterone were assayed by radioimmunoassay using rabbit antibodies, after extraction with ethyl acetate (20%) in hexane and celite column chromatography to eliminate binding proteins and cross-reacting substances (Nichols Institute, San Juan Capistrano, CA). Samples were assayed in five batches over a three month period (March to May) in 1991. To the extent possible, samples were distributed among batches to ensure that each batch included women from all strata of a cross-classification based on radiotherapy and ovariectomy status, time since treatment, and clinic where treatment occurred. For women who were subjected to repeat blood drawings, the paired samples were included in the same batch. The laboratory that conducted the assays was blinded to the ori-

gin of the samples. For a quality control pool from a postmenopausal woman, intra- and interassay coefficients of variation were 19% and 24% for estradiol, 14% and 12% for estrone, 19% and 20% for androstenedione, and 6% and 10% for testosterone. For a premenopausal quality control pool, corresponding values were 9% and 8% for estradiol, 11% and 10% for estrone, 14% and 18% for androstenedione, and 7% and 6% for testosterone. Assay sensitivities for the hormones were reported as 0.01 nmol/l (2 pg/ml) for estradiol, 0.04 nmol/l (10 pg/ml) for estrone, 0.10 nmol/l (30 pg/ml) for androstenedione, and 0.07 nmol/l (20 pg/ml) for testosterone. Estradiol measurements were below the sensitivity threshold for five women, two of whom were treated by irradiation, two by ovariectomy, and one by irradiation plus ovariectomy. The values reported by the laboratory, which ranged from 1.4 to 1.9 pg/ml, were used in the analysis.³

A questionnaire administered at the time of blood drawing addressed recent exogenous hormone use, recent smoking habits, height and weight, medical conditions, and surgical or radiotherapy procedures. Details about treatments for cervical cancer were obtained from medical records at the hospital where first diagnosis and treatment occurred. Radiation doses were estimated by medical physicists using procedures described by Stovall *et al.* (17).

Analysis. Linear regression methods (18) were used to evaluate associations between hormone concentrations and selected covariates. Natural logarithms of hormone concentrations were taken to stabilize the variances. Non-irradiated woman with one or both ovaries intact were chosen as the reference group. Most of these women had early-stage cervical cancer and were treated by hysterectomy (or cervical amputation) only; five women, all of whom were younger than 50 y at the time of phlebotomy, had unilateral ovariectomy. Two irradiated women also had had one ovary removed.

Ratios of adjusted mean hormone concentrations among women treated by ovariectomy, radiotherapy, or ovariectomy plus radiotherapy, relative to levels among hysterectomy-only patients (MRs) were estimated separately for women younger than 50 y at the time of phlebotomy and women age 50 or greater. Analyses were adjusted for age and time of day at phlebotomy (morning/afternoon), BMI at phlebotomy (kg/m²), and recent cigarette smoking, defined as having smoked one or more cigarettes earlier in the day on which blood samples were drawn (no/yes), using the model:

$$\begin{aligned} \ln [\text{hormone}] &= \beta_0 + \beta_1 \cdot \text{AGE} + \beta_2 \cdot \text{TIME} + \beta_3 \cdot \text{BMI} \\ &+ \beta_4 \cdot \text{SMOKE} + \beta_5 \cdot \text{OOPH} + \beta_6 \cdot \text{RAD} + \beta_7 \cdot \text{BOTH} \end{aligned}$$

where OOPH, RAD, and BOTH are 0/1 indicators of type of treatment namely, ovariectomy, radiotherapy, and ovariectomy and radiotherapy, respectively. MRs were estimated as $\exp(\beta_5)$, $\exp(\beta_6)$ and $\exp(\beta_7)$, respectively. MRs less than 1.00 indicate a lower mean concentration in the index group than in the reference group. Ninety-five % CIs were calcu-

³ Molar concentrations (nmol/l) are presented throughout the remainder of this paper; they can be converted to pg/ml by multiplying by the molecular weight, which is 272 g/mol for estradiol, 270 for estrone, 287 for androstenedione, and 288 for testosterone.

Table 2 Means and prevalences (%) for selected demographic, medical, anthropomorphic, and reproductive characteristics of cervical patients, separately by history of radiotherapy and ovarian status at the time of blood drawing.

	Bilateral Ovariectomy			
	No		Yes	
	Nonirradiated	Irradiated	Nonirradiated	Irradiated
Number of women	32	79	21	15
Age at cervical cancer diagnosis (y)	33	53	51	45
Cervical cancer stage = <i>in situ</i> (%)	37	3	5	7
Premenopausal at cancer diagnosis (%)	94	43	48	67
Age at blood draw (y)				
Mean	38	61	61	52
Minimum–maximum	25–66	26–86	42–76	36–68
Time since cancer diagnosis (y)	5	8	10	7
Hysterectomy (%)	94	6	100	93
Ever exogenous hormone use (%) ^a	16	25	43	33
Age at menarche (y)	12.9	13.1	12.9	11.9
Any children (%)	84	90	95	93
Age at first birth (y)	21	22	21	20
Current smoker (%) ^b	44	33	57	33
Weight at blood draw (kg)	66.5	69.0	69.5	64.3
BMI at blood draw (kg/m ²)	25.1	26.9	26.6	25.4
History of thyroid disorder (%)	6	14	19	7

^a Women who reported using exogenous hormones within the 2 weeks preceding the date of blood draw were excluded.

^b One or more cigarettes smoked on the day of blood draw.

lated as $\exp[\beta \pm 1.96 \text{ (standard error of } \beta)]$. For women with repeat blood draws, paired t-tests of untransformed pre- and post-treatment hormone concentrations were used.

Results

A cross-classification based on radiotherapy (no/yes) for cervical cancer and bilateral ovariectomy (no/yes) at time of blood draw defines four groups, for which selected characteristics are summarized in Table 2. Nonirradiated women with intact ovaries (reference group) were decidedly younger, both at time of cervical cancer diagnosis and at time of blood draw, than women who had been irradiated or surgically castrated. All but two of the women in the reference group were premenopausal at the time of cervical cancer diagnosis. Most women who had their ovaries removed also had a hysterectomy. However, the frequency of hysterectomy was low among irradiated women with intact ovaries. Sixteen % of the women in the reference group and 25–43% in the other three groups reported having taken exogenous hormones at some time in the past, although not within the preceding 2 weeks. Mean reported age at menarche was 12 or 13 y for all four groups, and other reproductive variables also were similar, although a smaller percentage of women in the reference group had had children. The prevalence of cigarette smoking at the time of phlebotomy was higher for nonirradiated women than for irradiated women. Mean weight and BMI (kg/m²) at time of phlebotomy varied by $\leq 8\%$ among groups. Among irradiated women, the average radiation dose to the ovaries was 50 Gy; dose to the adrenal glands was not estimated explicitly, but would have been similar to the kidney dose, about 2–3 Gy (Stovall *et al.*, 1989). Mean dose to the liver, where estrogens are metabolized prior to excretion, also would have been of the order of 2–3 Gy.

Unadjusted hormone concentrations in relation to age at phlebotomy for the reference group of women treated by hysterectomy only are shown in Figure 1. Hormone concentrations were negatively associated with age, except for

testosterone. For estradiol, the drop was abrupt and occurred in the mid- to late-40s, ages at which physiological menopause commonly occurs.⁴ Indeed, among women in the present study who reported having experienced spontaneous menopause prior to their diagnosis of cervical cancer, the average age at menopause was 46 y. A further decline in estradiol levels with age >45 y was not apparent. The wide variation in estradiol concentrations among women younger than 45, who, for the most part, were premenopausal, is partially attributable to the sample collection not having been standardized with respect to stage of menstrual cycle. A similar explanation may account for variations in concentration of the less cyclic estrogen, estrone. Concentrations of estrone and androstenedione declined more gradually with age. Slopes were significantly different from zero ($P_{(2)} < 0.01$) for androstenedione and estrone when evaluated over the entire range of age. Data for age ≥ 50 are too sparse to reveal whether the downward trends continued past the menopausal ages. For testosterone, a monotonic association with age was not apparent. Testosterone decreased in tandem with androstenedione for ages less than 45. At older ages, however, whereas the trend remained negative for androstenedione, the concentration of testosterone was positively associated with age. This result is not robust, as it is largely attributable to high testosterone values for two women, ages 53 and 65 (Fig. 1).

Radiotherapy and bilateral ovariectomy, alone or in combination, were associated with lower estrogen and androgen concentrations, relative to women who had undergone hysterectomy only, especially for estrogens at the younger ages (Table 3). The two types of treatment were associated with similarly reduced hormone levels, and,

⁴ Because these women had undergone hysterectomies, cessation of menstruation could not be used a marker of changes in ovarian function associated with menopause. Women were not assumed to have become postmenopausal based on hysterectomy alone.

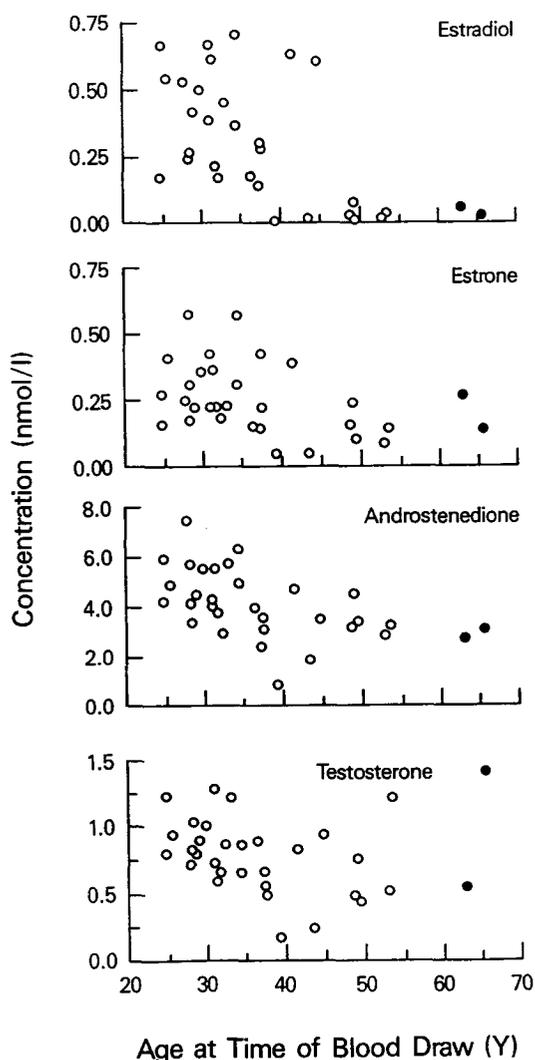


Fig. 1. Estrogen and androgen concentrations in relation to age at phlebotomy among nonirradiated women with intact ovaries who, for the most part, were treated by hysterectomy (see text). Open circles (○) denote women who were premenopausal at the time of diagnosis of cervical cancer; closed circles (●) represent those who were postmenopausal at the time of cervical cancer diagnosis. Two points were off the scale; the estradiol concentration for a 28-year-old woman was 1.33 nmol/l, and the estrone concentration for a 45-year-old woman was 0.91 nmol/l.

among women who had had both ovaries removed, radiotherapy seemed to reduce hormone levels only slightly, if at all. Differences among MRs for ovariectomy, radiotherapy, and ovariectomy plus radiotherapy were not significant ($P_{(2)} > 0.05$) for any of the hormones in either of the age categories. Because of this lack of heterogeneity by treatment, data for the three treatment groups were pooled (Table 4). In the aggregate data, strong evidence of heterogeneity in MR by age at phlebotomy was seen only for estradiol, with a much lower MR at younger ages. The MR for estrone also was lower for the younger age group, but the difference between age categories was less. Conversely, for testosterone, the point estimates for the MR for ovariectomy and/or radiotherapy were lower, though not significantly lower, for women age 50 y or older than for women younger than

Table 3 Ratio of adjusted mean hormone concentrations (MR) for women treated by radiotherapy, ovariectomy, and radiotherapy plus ovariectomy, relative to levels in nonirradiated women with intact ovaries, separately by age when blood sample was taken.

Sample sizes are given in Table 1. Note the small sample sizes for the hysterectomy only group age 50 y or older and for women treated by ovariectomy (with or without radiotherapy) at ages <50 (Table 1).

Hormone	Bilateral Ovariectomy	MR ^{a, b}			
		Radiotherapy			
		<50 y		≥50 y	
Estradiol	no	1.00	.20 ^c	1.00	.57 ^c
	yes	.13 ^c	.13 ^c	.51 ^c	.49 ^c
Estrone	no	1.00	.59 ^c	1.00	.71
	yes	.41 ^c	.49 ^c	.71	.59 ^c
Androstenedione	no	1.00	.73 ^c	1.00	.77
	yes	.87	.84	.77	.71
Testosterone	no	1.00	.85	1.00	.63
	yes	1.11	.83	.49 ^c	.40 ^c

^a Adjusted for age at phlebotomy, BMI, recent smoking, and time of day of phlebotomy. Adjusting for hospital where treatment for cervical cancer occurred or usual alcohol consumption (per previous mail questionnaire) did not change estimates appreciably.

^b Reference category, neither radiotherapy nor bilateral ovariectomy.

^c $P_{(1)} < 0.05$ for test of null hypothesis that $MR \geq 1.00$.

Table 4 Estimates of ratios of mean hormone concentrations (MR) for women treated by radiotherapy and/or ovariectomy, relative to levels in nonirradiated women with intact ovaries, separately by age when blood sample was taken

Hormone	MR ^a		MR ^a		Test for heterogeneity $P_{(2)}$
	<50 y		≥50 y		
	MR ^a	95% CI	MR ^a	95% CI	
Estradiol	0.17	0.09–0.31	0.55	0.32–0.95	<0.01
Estrone	0.54	0.37–0.81	0.69	0.44–1.09	0.11
Androstenedione	0.77	0.57–1.04	0.76	0.47–1.23	0.88
Testosterone	0.86	0.64–1.15	0.57	0.32–0.99	0.42

^a Pooled estimate for radiotherapy, ovariectomy and radiotherapy plus ovariectomy groups relative to hysterectomy only patients.

50 y. The MR for androstenedione was nearly equal among women younger than 50 y and those age 50 y or older.

To give an idea of the absolute differences associated with these ratio measures, fitted mean concentrations by treatment category for a nonsmoking woman age ≥50 y and of average body mass (26.1 kg/m²) are shown in Fig. 2. Although radiotherapy and ovariectomy were associated with large relative differences in estradiol concentration, as reflected in the MRs, the absolute differences among the women older than 50 were small; estradiol concentrations were very low even among the hysterectomy-only women who, presumably, had experienced physiological menopause. Furthermore, these low levels are approaching the sensitivity threshold for the assay.

Hormone concentrations were not associated with radiation dose to the ovaries for the radiotherapy-only group. Doses to the ovaries were almost uniformly large, with 95% of the women receiving ≥38 Gy.

Repeat Samples. Pre- and post-treatment (4–12 months) blood samples were available for nine women who were treated by radiotherapy only and who did not take exogenous estrogens in the interim; five women were premeno-

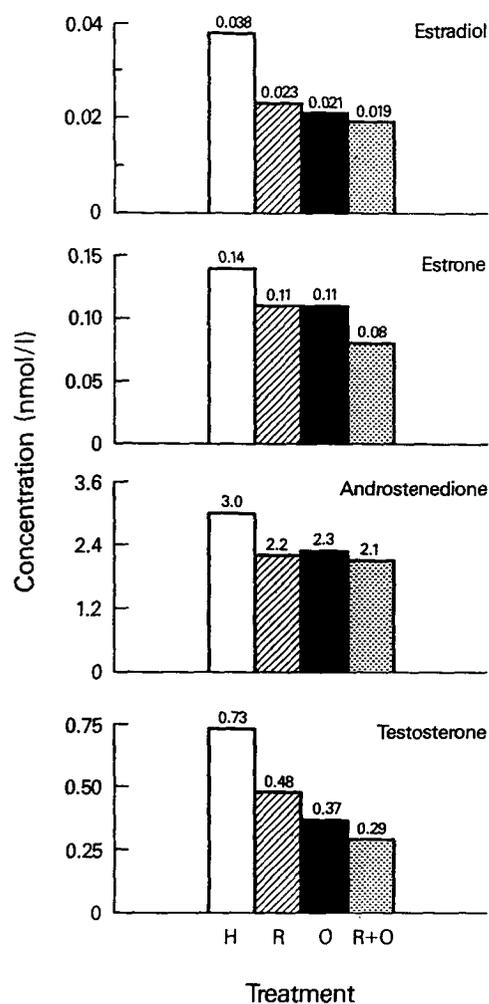


Fig. 2. Fitted mean hormone concentrations for a nonsmoking woman of average BMI (26.1 kg/m^2) and \geq age 50 years at phlebotomy, assuming treatment by hysterectomy (H), radiotherapy (R), ovariectomy (O) or radiotherapy + ovariectomy (R + O).

pausal at the time of cervical cancer diagnosis (mean age, 48 y), and four were postmenopausal (mean age, 67 y). Only the post-treatment measurements were used in the preceding analyses and illustrations. With regard to the premenopausal women, it should be noted that sample collection was not done at a specified point in the menstrual cycle. Average concentration differences (after–before) were negative for all four hormones among pre- and postmenopausal women (Figure 3 and Table 5). Decreases were significant only for estradiol among premenopausal women and for testosterone among postmenopausal women (Table 5). Among women age 55 and over, testosterone concentrations were consistently lower after radiotherapy than before. Similar results were obtained for androstenedione, except for one 85-year-old woman. Estrogen concentrations among women over age 55 changed only slightly following irradiation and not in a consistent direction.

Discussion

Ovariectomy and high-dose pelvic radiotherapy (mean dose to ovaries, 50 Gy) were associated with reduced serum con-

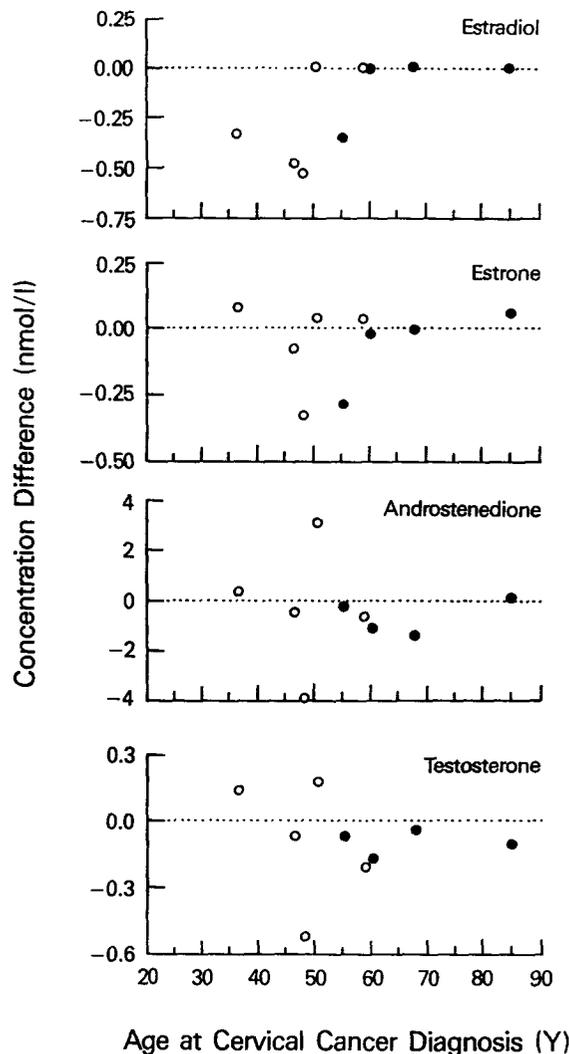


Fig. 3. Difference in hormone concentrations (after–before) among irradiated women for whom blood samples were collected prior to treatment for cervical cancer, and 4–12 months after treatment. Women who were premenopausal at the time of cervical cancer diagnosis are represented by open circles (O) and those who were postmenopausal by closed circles (●). One 59-year-old woman evidently was still premenopausal at the time of cervical cancer diagnosis.

centrations of estrogens and androgens relative to levels in nonirradiated women with intact ovaries, even among postmenopausal women and many years after treatment. Radiotherapy plus ovariectomy was associated with slightly lower concentrations than either treatment alone, but the differences appeared to be negligible, indicating that the two types of treatment have similar effects on serum hormone levels.

If irradiation were suppressing both ovarian and adrenal production of androgens, one would have expected radiotherapy to have an effect beyond that associated with ovariectomy. Only weak evidence for this was seen. Like Eby *et al.* (16), we observed slightly, but not significantly, lower concentrations of androgens and estrogens associated with radiotherapy among postmenopausal women lacking ovaries. Given that: (i) MRs were not significantly lower for

Table 5 Pretreatment estrogen and androgen concentrations and changes in concentration among irradiated cervical cancer patients with intact ovaries, separately by menopausal status at the time of cervical cancer diagnosis.

Sample collection from premenopausal women was not standardized with respect to stage of menstrual cycle.

Menopausal status at cervical cancer diagnosis	Number of patients	Hormone	Average concentration (nmol/l)		
			Before treatment	Difference (after-before)	$P_{(t)}$ ^a
Premenopausal ^b	5	estradiol	0.31	-0.27	0.04
		estrone	0.24	-0.05	0.25
		androstenedione	3.31	-0.34	0.39
		testosterone	0.63	-0.10	0.24
Postmenopausal ^c	4	estradiol	0.10	-0.09	0.20
		estrone	0.16	-0.06	0.23
		androstenedione	2.36	-0.64	0.08
		testosterone	0.70	-0.10	0.02

^a Paired t-test of difference between untransformed pre- and post-treatment hormone concentrations.

^b Average age at first blood draw, 48 y; average interval between samples, 5.1 months.

^c Average age at first blood draw, 67 y; average interval between samples, 8.2 months.

women who had radiotherapy in addition to bilateral ovariectomy, (ii) the average treatment dose to the adrenal glands was 2–3 Gy, as contrasted with a dose of 50 Gy to the ovaries, and (iii) the adrenal gland is believed to be relatively radioresistant (19) and to have substantial reserve capacity that can be mobilized in the event of damage (20), the case for significant adrenal suppression associated with radiotherapy for cervical cancer is weak. Greater support can be mustered for the view that high-dose irradiation, as well as ovariectomy, eliminates ovarian sources of androgen production in postmenopausal women. Although radiation suppression of the adrenal gland cannot be ruled out, such an effect did not seem to be quantitatively important relative to the effect on the ovary.

Because of the cross-sectional nature of these data, we do not know whether hormone levels were reduced in all women undergoing these treatments or only in certain subgroups. For example, Procopé and Adlercreutz (21) reported decreases in urinary estrone and estradiol following bilateral ovariectomy among postmenopausal women with ovarian cortical stromal hyperplasia, whereas little change was seen among women with histologically atrophic ovaries. In the present study, pre- and postradiotherapy sampling for a small group of postmenopausal women tended to corroborate cross-sectional findings for the androgens, but results for estrogens were ambiguous. On average, estrogen concentrations also declined, but this was driven by a rather large decrease for one woman, while values for the other three postmenopausal women changed little.

Differences among MRs for the four hormones and between women younger than 50 y and those over 50 likely reflect, in part, differences in the ovarian contribution to circulating hormone levels. For example, ovarian secretion of estrogens changes dramatically with age. Among premenopausal women, the ovaries are of central importance, but among postmenopausal women, the ovaries secrete little estrogen. The principal source of circulating estrogen among postmenopausal women is believed to be extraglandular conversion (aromatization) of androstenedione to estrone (12, 13). Although persistent ovarian production of small quantities of estradiol has been detected among some postmenopausal women (10, 22, 23), perhaps those in whom follicles do not disappear completely (24), most estradiol is believed to originate peripherally, via reduction of estrone and, to a lesser extent, aromatization of testosterone (25–27).

In most cases, the postmenopausal ovary is thought to have lost the capacity to aromatize androgens into estrogens (28). The lower estradiol MRs associated with ovariectomy or radiotherapy for age at phlebotomy <50 y, as contrasted with age ≥50, can be explained by the elimination or reduction of estradiol secretion by premenopausal ovaries. At older ages, the ovarian contribution to plasma estradiol is reduced substantially and ovarian ablation has a lesser relative effect.

Both the ovary and the adrenal gland contribute to the pool of circulating androgens, although the relative magnitudes of contributions from these two sources vary with age and menopausal status (11, 22, 29–32). Secretion of androstenedione by both the ovary and the adrenal are lower postmenopausally than premenopausally, but the relative decline appears to be greater for the ovary (30, 33). Whereas the follicle, corpus luteum, and ovarian stroma all produce appreciable quantities of androstenedione in premenopausal women (34), only the stroma persists in postmenopausal women. Among postmenopausal women, most androstenedione is of adrenal origin (30, 35, 36). Based on measurements in women before and after ovariectomy, Judd *et al.* (9) estimated that ovarian secretion accounts for about 50% of plasma androstenedione among premenopausal women, but only about 20% among postmenopausal women. In the present study, (data shown in Table 4), the ovarian contribution to circulating levels of androstenedione might be estimated as 20–25% for pre- as well as postmenopausal women. The difference between our results and those of Judd *et al.* could easily be due to chance.

Interpreting MRs in this way does not account for possible compensatory or other physiological or pathological responses to treatment. Abraham *et al.* (37) reported that the metabolic clearance rate for testosterone decreased following surgical menopause. Grattarola (38) noted that production of adrenal androgens is under the control of gonadotropins, and that pituitary gonadotropins increase following ovariectomy. Levels of urinary estrogens increased following administration of corticotropin to ovariectomized women (21), illustrating the possible mediating role of the pituitary, the reserve capacity of the adrenal gland, and its likely importance as an indirect source of estrogens in postmenopausal women.

Among premenopausal women, most (50–60%) plasma testosterone appears to derive from peripheral conversion of secreted androstenedione, and the remainder by direct se-

cretion from the adrenal gland or ovary (39, 40). Testosterone levels among women in the reference group declined in parallel with androstenedione levels between the ages of 20 and 45 y, probably reflecting an age-related decline in secretion of androstenedione (35). However, unlike androstenedione, testosterone concentrations did not continue to decrease during and after the menopausal ages. Whereas ovarian secretion of androstenedione seems to decrease following menopause, the relative and absolute ovarian contribution to plasma testosterone seem to increase following menopause, at least temporarily, perhaps in response to stimulation by rising levels of leutinizing hormone, which accompany or follow the menopausal decline in estrogens (9, 10, 22, 41). Testosterone production in the postmenopausal ovary occurs in the stroma under the positive regulation of leutinizing hormone (32, 42, 43). The ovarian contribution to plasma testosterone in postmenopausal women has been estimated to be of the order of 50%, with the adrenal gland accounting for most of the remainder (11, 28). In the present study, among women age 50 or older, ovariectomy and radiotherapy were associated with testosterone concentrations approximately 45% below those of women treated by hysterectomy only. However, among women younger than 50, the difference was only about 15%. These findings fit with the view that the ovaries account for a greater fraction of circulating testosterone in postmenopausal women. Consistent with the idea that the ovary is a relatively minor contributor to plasma testosterone in premenopausal women is the finding of Janson *et al.* (44) that although the onset of radiotherapy for cervical cancer was accompanied by immediate and marked decreases in estradiol concentrations, there was little change in testosterone levels.

The very high radiation doses associated with radiotherapy for cervical cancer probably sufficed to eliminate ovarian production of androgens, as well as estrogens. However, it is possible that considerably lower doses, although still sufficient to suppress estrogen production and induce menopause in premenopausal women, might leave the androgen-producing stroma substantially intact. Several observations indicate that stromal cells, the sites of much of this production, are more radioresistant than the estrogen-producing granulosa cells (45–47).

Methodologic Issues. Determinants of type of treatment for cervical cancer might be related to hormonal levels. For example, women treated by hysterectomy alone usually had early-stage disease at first diagnosis, and such a group might differ from women diagnosed later in the natural history. Fraser *et al.* (48) reported an elevated rate of production of estradiol in a small sample of premenopausal cervical cancer patients with early-stage disease relative to healthy controls. It also is possible that hysterectomy influenced ovarian function (49). Siddle *et al.* (50) reported that hysterectomy seemed to hasten the time of ovarian failure among premenopausal women, and the mean age at natural menopause among cervical cancer patients in the present study seemed to be several years younger than among women in the United States in general. Although such a phenomenon might limit generalizability of our findings to healthy women with intact ovaries, it would not account for the observed negative associations between hormone levels and ovariectomy or radiotherapy. Furthermore, age-specific estrogen and androgen measurements among cervical cancer patients treated by hysterectomy were generally similar to published values for healthy women with intact ovaries (10, 51–

53), although Judd *et al.* (51) and Meldrum *et al.* (53) found somewhat lower values for androstenedione. It would seem that hysterectomy by itself did not radically or permanently depress steroid hormone levels, apart from a possible effect in advancing the age of menopause, and that nonirradiated cervical cancer patients with intact ovaries are not grossly different from healthy women with respect to hormone profiles.

There were only four women in the reference group over age 50 at the time of blood draw and just two younger than 50 y in the ovariectomy alone category. Estimates of effect (MR) were sensitive not only to random variation in hormone levels among women but also to possible misclassification of even one or two women with respect to ovarian status, exogenous estrogen use, or hormone level. Furthermore, age at menopause was unknown for most of the women in the reference category. Vermeulen (54) reported that ovaries can continue to contribute appreciably to plasma estrogen levels during the first several years following menopause, which raises the possibility that results for the reference group are influenced by inclusion of women in their perimenopausal years. However, restriction of our analysis to include only women older than 50 y and two or more years post-treatment did not change the MRs appreciably, nor did we observe gradients in estrogen concentrations with age among women in the reference group who were older than 45 y at the time of phlebotomy. Thus, estimates of effect were not particularly sensitive to an arbitrary decision about what age to use in separating women by menopausal status. Nonetheless, the small number of women in the reference group older than 50 years remains a serious limitation. Furthermore, the imbalanced age distribution across the four treatment groups precluded us from addressing, in an informative way, the issue of heterogeneity of effect with age or time.

Plasma hormone levels in postmenopausal women are variable even on a time scale of hours, as secretion and metabolism can be episodic (11, 55); a single blood sample might not suffice to characterize the usual hormone profile for a given woman. An additional possible problem is that samples were stored for 5–6 y prior to being assayed, and at -20° rather than -70° . Although concentrations of sex hormones in frozen samples are said to remain stable for 15 y if containers are tightly sealed (56, 57), the possibility of deterioration cannot be dismissed. However, several observations indicate that these limitations were not so serious as to vitiate the data. The age-specific hormone concentrations that we observed among women treated by hysterectomy only varied with age in a regular fashion and are consistent with the literature. Other widely-reported epidemiological associations also were seen, such as those between BMI and estrogen levels (58), cigarette smoking and androstenedione concentration among postmenopausal women (57), and time of day of sampling and sex hormone concentration (11). Finally, although women known to have used exogenous estrogens in the days or weeks preceding donation of blood were excluded from this paper, their blood samples were assayed and found to have higher estradiol and estrone concentrations than other women in the same treatment group. Thus, the overall pattern of the results seems to be orderly rather than random, which indicates that artifacts of sampling were not responsible for the observed patterns.

Summary. Radiotherapy and bilateral ovariectomy both were associated with reduced concentrations of circulating estrogens and androgens in comparison to levels observed

among women treated by hysterectomy only, even when the analysis was restricted to women age 50 or older. In most cases, the relative decreases were similar for radiotherapy only and ovariectomy only groups. Evidence that irradiation further reduces hormone levels beyond the reduction attributable to ablation of ovarian function is weak. Although the possibility of a radiation effect on the adrenal gland or other extraovarian tissue cannot be dismissed, it is not necessary to invoke such extraovarian targets to explain these results. Results are compatible with the idea that the two types of treatment are influencing the same endocrine tissues, in the one case by killing or inactivating the secretory cells and in the other by removing them altogether. Insofar as the normal postmenopausal ovary has lost most, if not all, of its ability to produce estrogens (28, 54, 59), a reasonable inference is that the relevant cells are the androgen-secreting cells of the ovarian stroma. Suppression of androgen secretion by the postmenopausal ovary could influence the concentration of estrone and estradiol as well, by reducing the pool available for peripheral aromatization. It is not clear whether the changes in androgen concentrations are of any clinical significance. Although the present study does not directly address the relationship between breast cancer risk and pelvic irradiation, when coupled with previous reports of a reduced risk of breast cancer associated with radiotherapy for cervical cancer (14), even among women irradiated postmenopausally, results indicate the possible continuing importance of ovarian hormone production among postmenopausal women as a causal factor at one or more stages of the disease process.

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