

# High Testosterone and Low Progesterone Circulating Levels in Premenopausal Patients with Hyperplasia and Cancer of the Breast<sup>1</sup>

Giorgio Secreto,<sup>2</sup> Camilla Recchione, Giuseppe Fariselli, and Sergio Di Pietro

Laboratorio di Ricerca Ormonale [G. S., C. R.] and Oncologia Clinica A [G. F., S. D.], Istituto Nazionale per lo Studio e la Cura dei Tumori, Milan, Italy

## ABSTRACT

Serum testosterone, progesterone, luteinizing hormone, prolactin, and sex hormone-binding globulin levels were measured in 55 normal controls, in 31 patients with hyperplastic alterations of breast epithelium, and in 23 patients with breast cancer. All patients and controls were premenopausal, and they were comparable for age, weight, and body surface. In the controls, the mean level of testosterone [ $0.47 \pm 0.16$  (S.D.) ng/ml] was lower and the mean level of progesterone [ $17.63 \pm 8.11$  ng/ml] was higher than in breast cancer patients [testosterone level,  $0.62 \pm 0.22$  ng/ml ( $p < 0.005$ ); progesterone level,  $11.4 \pm 8.0$  ng/ml ( $p < 0.005$ )] and in patients with breast epithelial hyperplasia [testosterone level,  $0.55 \pm 0.2$  ng/ml ( $p \leq 0.05$ ); progesterone level,  $13.9 \pm 8.6$  ng/ml ( $p < 0.05$ )]. No difference was found in the mean circulating levels of luteinizing hormone, prolactin, or sex hormone-binding globulin between controls and patients. These results confirm previous findings of increased urinary testosterone excretion in women with anovulatory menstrual cycles and epithelial hyperplasia or cancer of the breast and strongly support the hypothesis that androgens play an important role in both induction and development of breast cancer.

## INTRODUCTION

In a previous paper (23), we found serum testosterone levels to be significantly higher than normal in postmenopausal breast cancer patients. This finding supported previous reports of increased urinary testosterone excretion in these patients (10, 11). Increased urinary testosterone excretion was also found in premenopausal breast cancer patients (8, 22) and in patients with hyperplastic alterations of breast epithelium (9), suggesting that androgens play an important role in induction and development of breast cancer. In these patients, the increased urinary androgen excretion was shown to be associated with anovulatory menstrual cycles (8, 9).

Blood concentrations of hormones are considered better parameters of their biological activity than their urinary excretion. The purpose of this study was, consequently, to support previous findings in urine (8, 9, 22) by determining the circulating levels of testosterone, progesterone, LH,<sup>3</sup> prolactin, and SHBG in premenopausal women with epithelial hyperplasia or cancer of the breast.

## MATERIALS AND METHODS

Fifty-five normal controls, 31 patients with histologically proven hyperplastic alterations of breast epithelium, and 23 patients with a histological diagnosis of infiltrating carcinoma of the breast entered the study. Controls were selected from healthy women according to the following criteria: no abnormality of the breast at the clinical examination; no benign or malignant neoplasias outside of the breast; and no endocrine, metabolic, or chronic diseases. Patients with breast epithelial hyperplasia were examined within 6 months after biopsy, and breast cancer patients were examined before mastectomy. All of the subjects in the study were premenopausal, and none of them was taking any hormonal drug for at least 6 months before hormonal examination or digitalis, antihypertension drug, or psychodrugs for chronic disease.

Hormone determinations were made on peripheral blood samples drawn between 9 a.m. and 11 a.m. on Days 20 to 23 of the menstrual cycle. The collected blood was allowed to clot at room temperature and was centrifuged; the serum was separated and stored at  $-20^{\circ}$  until the assay. All of the hormones and SHBG were determined in the same blood samples.

Testosterone was quantitatively extracted from serum with diethyl ether (spectrograde Merck) and measured by radioimmunoassay according to the method of Ismail *et al.* (14). <sup>125</sup>I-labeled testosterone-3-tyrosine-methyl-ester and specific antiserum against testosterone-3-carboxymethyloxime-bovine serum albumin were used for the assay. The separation of the free antigen from the complex was performed by the polyethylene glycol method (3).

LH and prolactin were respectively determined with the method of Midgley (18) and Sinha *et al.* (27). LH was expressed according to the second International Reference Preparation of Human Menopausal Gonadotropin, and prolactin was expressed in ng according to the NIH Standard (NIH Code F1) ( $1 \text{ ng} = 23 \pm 3 \mu\text{IU}$  of WHO 71/222). Testosterone-3-tyrosine methyl ester, LH, and prolactin, labeled with <sup>125</sup>I by use of the chloramine-T method (12), and the respective antibodies were supplied by Biodata (Milan, Italy).

Radioimmunoassay of progesterone was performed without preliminary extraction by the method of El Shami *et al.* (4). The progesterone <sup>125</sup>I-11-tyrosine and the specific antiserum against progesterone 11-HSA, immobilized to the wall of the polypropylene tubes, were obtained from Diagnostic Products Corporation (Los Angeles, CA).

SHBG was quantitated by its ability to bind DHT. The binding protein saturated with  $[1,2\text{-}^3\text{H}]\text{DHT}$  was separated by the ammonium sulphate technique of Rosner (21).  $[1,2\text{-}^3\text{H}]\text{DHT}$  was purchased from Biodata (Milan, Italy).

The coefficients of intra- and interassay variation were, respectively, 5 and 12% for testosterone, 5.5 and 7% for LH, 5.86 and 9% for prolactin, 6 and 13% for progesterone, and 2.7 and 5.2% for SHBG. Comparisons of testosterone, progesterone, prolactin, and SHBG levels between the control group, cancer group, and hyperplasia group were performed by Student's *t* test after conversion of data to a logarithmic scale for prolactin and SHBG; testosterone and progesterone values were processed without log transformation of the data. Significance between groups was confirmed by variance analyses. Comparisons of LH data were performed using a nonparametric test (Mann-Whitney)

<sup>1</sup> Supported in part by Grant 82.01335.96, Progetto Finalizzato "Controllo della Crescita Neoplastica" from the Consiglio Nazionale delle Ricerche, Rome, Italy.

<sup>2</sup> To whom requests for reprints should be addressed, at Istituto Nazionale Tumori, Via Venezian 1, 20133 Milan, Italy.

<sup>3</sup> The abbreviations used are: LH, luteinizing hormone; SHBG, sex hormone-binding globulin; DHT, dihydrotestosterone.

Received May 3, 1983; accepted October 26, 1983.

because of the nonhomogeneity of variances. Two-tailed  $p$  values were considered for statistical analyses;  $p \leq 0.05$  was considered significant.

## RESULTS

The 3 groups of subjects were comparable for age, age at menarche, marriage and first full-term pregnancy, number of pregnancies, height, weight, and body surface. Table 1 reports the ranges and means for the various characteristics of the patients and controls.

In the controls, the mean circulating levels of testosterone [ $0.47 \pm 0.16$  (S.D.) ng/ml] were significantly lower and the mean levels of progesterone ( $17.63 \pm 8.11$  ng/ml) were significantly higher than in breast cancer patients [testosterone level,  $0.62 \pm 0.22$  ng/ml ( $p < 0.005$ ); progesterone level,  $11.4 \pm 8.0$  ng/ml ( $p < 0.005$ )] and in patients with breast epithelial hyperplasia [testosterone level,  $0.55 \pm 0.2$  ng/ml ( $p \leq 0.05$ ); progesterone level,  $13.9 \pm 8.6$  ng/ml ( $p < 0.05$ )]. Neither testosterone nor progesterone levels differed significantly between the 2 groups of patients (Table 2, Charts 1 and 2).

No difference was found among controls, patients with breast epithelial hyperplasia, and patients with breast cancer in the mean levels of LH, prolactin, or SHBG (Table 2, Charts 3 to 5).

## DISCUSSION

Detection of circulating levels of testosterone that were higher than normal in premenopausal breast cancer patients supports previous reports from our laboratory (8, 22) of increased urinary testosterone excretion. Our findings are in agreement with the data of Malarkey *et al.* (16), who reported 24-hr mean serum concentrations of testosterone that were significantly higher than normal in breast cancer patients examined before mastectomy in the luteal phase of their menstrual cycle. We were unable to find any other report of blood testosterone levels in premeno-

pausal patients with breast cancer. The few reports on postmenopausal patients are conflicting; normal values of testosterone have been found by some authors (2, 13, 16) and increased levels by others (1, 17) and by ourselves (23).

In our series, breast cancer patients showed progesterone levels that were significantly lower than normal. This finding fits very well with the anovulatory hypothesis of Grattarola (7) and with the inadequate corpus luteum function hypothesis of Sherman and Korenman (24), and it is in agreement with the data of Kodama *et al.* (15) of subnormal urinary excretion of pregnenediol, the principal metabolite of progesterone. In contrast, normal progesterone levels have been found by others (5, 6, 29) in the blood of patients with breast cancer.

The circulating levels of prolactin, LH, and SHBG did not differ

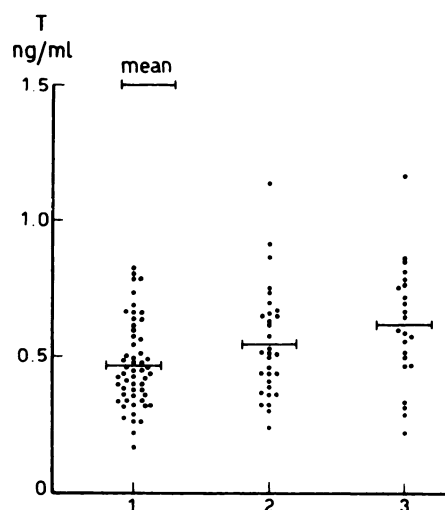


Chart 1. Individual values of testosterone (T) in normal controls (Group 1) and in patients with epithelial hyperplasia (Group 2) or cancer (Group 3) of the breast.

Table 1  
Characteristics of normal controls and of patients with hyperplasia or cancer of the breast

Patient characteristics	Controls			Breast hyperplasia			Breast cancer		
	No. of cases	Range	Mean	No. of cases	Range	Mean	No. of cases	Range	Mean
Age (yr)	55	27-53	39.09 $\pm$ 5.9 <sup>a</sup>	31	31-50	41.58 $\pm$ 5.3	23	29-52	41.7 $\pm$ 5.9
Age at menarche (yr)	53	10-16	13.03 $\pm$ 1.4	31	10-15	13.02 $\pm$ 1.3	23	11-15	13.09 $\pm$ 1.8
Age at marriage (yr)	47	18-33	24.04 $\pm$ 3.2	28	16-31	24.53 $\pm$ 4.3	21	19-34	23.85 $\pm$ 3.6
Age at first pregnancy (yr)	45	19-38	27.58 $\pm$ 5.3	24	19-35	26.27 $\pm$ 4.1	19	20-35	26.44 $\pm$ 4.1
No. of full-term pregnancies	47	0-6	1.63 $\pm$ 0.8	28	0-5	1.71 $\pm$ 1.2	21	0-6	1.95 $\pm$ 1.3
Height (cm)	52	147-173	160.3 $\pm$ 5.9	29	145-170	161.2 $\pm$ 6.7	22	148-172	160.6 $\pm$ 6.5
Wt (kg)	54	34-85	57.9 $\pm$ 8.6	30	40-79	56.1 $\pm$ 9.4	22	48-71	59.2 $\pm$ 6.2
Body surface (sq m)	51	1.34-1.92	1.6 $\pm$ 0.12	29	1.35-1.83	1.58 $\pm$ 0.14	22	1.44-1.77	1.61 $\pm$ 0.1

<sup>a</sup> Mean  $\pm$  S.D.

Table 2  
Circulating levels of testosterone, progesterone, prolactin, LH, and SHBG in controls and in patients with hyperplasia or cancer of the breast

	Controls			Breast hyperplasia			Breast cancer		
	No. of cases	Range	Mean	No. of cases	Range	Mean	No. of cases	Range	Mean
Testosterone (ng/ml)	55	0.17-0.82	0.47 $\pm$ 0.16 <sup>a</sup>	31	0.24-1.14	0.55 $\pm$ 0.2 <sup>b</sup>	23	0.22-1.17	0.62 $\pm$ 0.22 <sup>c</sup>
Progesterone (ng/ml)	55	0.10-39.70	17.63 $\pm$ 8.11	31	1.00-32.7	13.90 $\pm$ 8.6 <sup>b</sup>	23	0.10-29.8	11.4 $\pm$ 8.0 <sup>c</sup>
Prolactin (ng/ml)	54	5.50-53.5	12.78 $\pm$ 7.90	31	5.70-57.1	15.8 $\pm$ 10.5	22	4.20-36.8	13.3 $\pm$ 7.9
LH (mIU/ml)	39	6.90-58.0	15.31 $\pm$ 8.80	28	5.30-50.7	16.86 $\pm$ 10.2	14	8.00-93.4	22.3 $\pm$ 25.2
SHBG ( $\mu$ g of DHT/100 ml)	42	1.36-3.34	2.04 $\pm$ 0.48	23	1.47-4.09	2.33 $\pm$ 0.61	14	1.30-3.68	2.13 $\pm$ 0.78

<sup>a</sup> Mean  $\pm$  S.D.

<sup>b</sup> Significance versus controls;  $p < 0.05$ .

<sup>c</sup> Significance versus controls;  $p < 0.005$ .

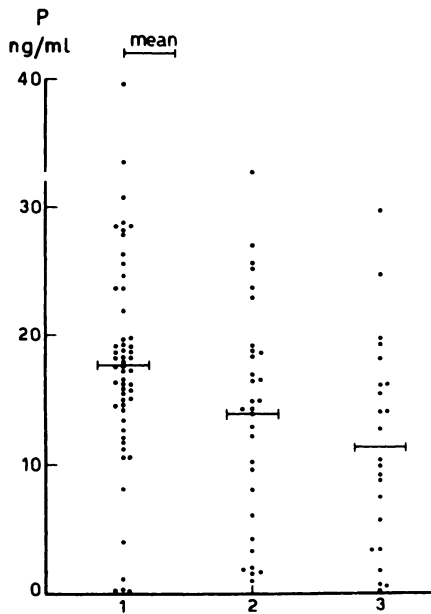


Chart 2. Individual values of progesterone (P) in normal controls (Group 1) and in patients with epithelial hyperplasia (Group 2) or cancer (Group 3) of the breast.

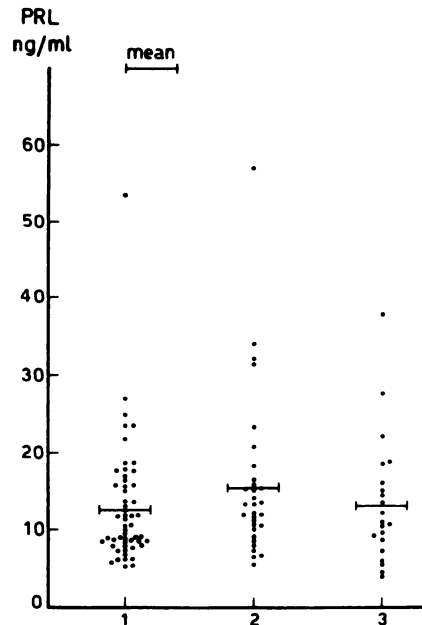


Chart 4. Individual values of prolactin (PRL) in normal controls (Group 1) and in patients with epithelial hyperplasia (Group 2) or cancer (Group 3) of the breast.

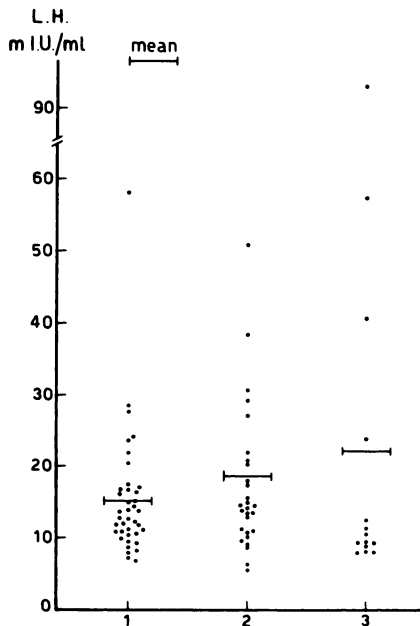


Chart 3. Individual values of LH (L.H.) in normal controls (Group 1) and in patients with epithelial hyperplasia (Group 2) or cancer (Group 3) of the breast.

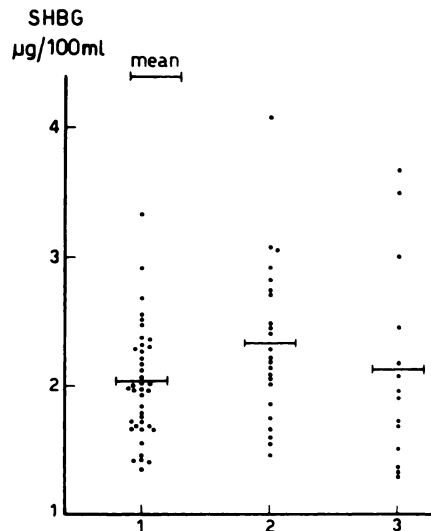


Chart 5. Individual values of SHBG in normal controls (Group 1) and in patients with epithelial hyperplasia (Group 2) or cancer (Group 3) of the breast.

from normal levels in our patients. Normal values of prolactin (6, 25) have been reported in the literature. The only 2 papers concerning LH levels in premenopause are conflicting; Malarkey *et al.* (16) found normal values, and Zumoff *et al.* (30) found significantly subnormal values. Both authors determined the mean 24-hr blood concentrations of the hormone. Our data, based on a single spot determination, are not entirely comparable, because of the wide diurnal variations of LH; nevertheless, in our series, nearly three-fourths of the cancer patients had clearly subnormal LH values (Chart 5), almost suggesting the existence of 2 populations. SHBG binding capacity has been

insufficiently studied in breast cancer: Moore *et al.* (19) recently found normal SHBG values in premenopausal patients and decreased values in postmenopausal patients.

Hyperplastic alterations of breast epithelium are a well-known factor of risk for breast cancer (20). In these patients, we found the same hormonal abnormality that we found in breast cancer patients, namely increased testosterone levels and decreased progesterone levels, which confirms previous findings in urine (9). The hormonal pattern of women with breast noncancerous diseases has been extensively studied, but many different histological findings, varying from cystic disease to fibroadenosis to benign adenoma, etc., have usually been considered together. Consequently, data from the literature are not entirely comparable with the findings of this study, in which patients have been

rigidly selected according to the histological diagnosis of epithelial hyperplasia. Malarkey *et al.* (16) reported normal values of testosterone and progesterone in patients with benign breast disease, and England *et al.* (5) found normal values of progesterone in patients with cystic disease as well as in patients with fibroadenosis of the breast. In contrast, Sitruk-Ware *et al.* (28), on the basis of subnormal progesterone levels, cited inadequate corpus luteum function as the hormonal alteration of fibrocystic disease of the breast.

In a previous paper (23), we reported circulating testosterone levels that were higher than normal in postmenopausal breast cancer patients; the same abnormal hormonal pattern, together with low progesterone levels, has been found in this study in premenopausal breast cancer patients and in women at enhanced risk for breast epithelial hyperplasia. Differences between patients and controls were not due to differences in body weight or body surface. Increased testosterone levels were present, along with normal levels of circulating SHBG, suggesting that there is an increased bioavailability of free testosterone in these patients. The supposed free testosterone excess could act with a double mechanism, *i.e.*, direct stimulation of the target tissue through binding to androgen receptors and increased metabolism into estradiol. From this point of view, our data could be in agreement with recent findings of increased free estradiol levels in breast cancer patients (19, 26).

In conclusion, our data on serum concentrations of testosterone in patients with breast cancer or with breast epithelial hyperplasia strongly support the hypothesis that androgens play an important role in induction and development of breast cancer. Further studies, mainly on the circulating levels of free steroids, will be necessary to better elucidate this role.

## ACKNOWLEDGMENTS

We thank Professor Antonio Liverani of the Centro di Calcolo of the University of Milan and Dr. Paola Pisani of the Epidemiology Service of the Istituto Nazionale Tumori for assistance with the statistical calculations and B. Johnston for editing and preparing the manuscript.

## REFERENCES

- Adami, H.-O., Johansson, E. D. B., Vegelius, J., and Victor, A. Serum concentrations of estrone, androstenedione, testosterone, and sex-hormone-binding globulin in postmenopausal women with breast cancer and in age-matched controls. *Ups. J. Med. Sci.*, 84: 259-274, 1979.
- Borkowski, A., L'Hermite, M., Dor, P., Longeval, E., Rozencweig, M., Muquardt, C., and Van Cauter, E. Steroid sex hormones and prolactin in postmenopausal women with generalized mammary carcinoma during prolonged dexamethasone treatment. *J. Endocrinol.*, 73: 235-246, 1977.
- Desbuquois, B., and Aurbach, G. D. Use of polyethylene glycol to separate free and antibody-bound peptide hormones in radioimmunoassays. *J. Clin. Endocrinol. Metab.*, 33: 732-738, 1971.
- El Shami, A. S., Coombes, R. F., Ziering, S., and Aroesty, S. A. Direct no-extraction assay for serum progesterone by solid phase  $^{125}\text{I}$  radioimmunoassay. *Clin. Chem.*, 28: 1613-1614, 1982.
- England, P. C., Skinner, L. G., Cottrell, K. M., and Sellwood, R. A. Sex hormones in breast disease. *Br. J. Surg.*, 62: 806-809, 1975.
- Franks, S., Ralphs, D. N. L., Seagroatt, V., and Jacobs, H. S. Prolactin concentrations in patients with breast cancer. *Br. Med. J.*, 4: 320-321, 1974.
- Grattarola, R. The premenstrual endometrial pattern of women with breast cancer. A study of progestational activity. *Cancer (Phila.)*, 17: 1119-1122, 1964.
- Grattarola, R. Androgens in breast cancer. I. Atypical endometrial hyperplasia and breast cancer in married premenopausal women. *Am. J. Obstet. Gynecol.*, 116: 423-428, 1973.
- Grattarola, R. Anovulation and increased androgenic activity as breast cancer risk in women with fibrocystic disease of the breast. *Cancer Res.*, 38: 3051-3054, 1978.
- Grattarola, R., Secreto, G., Recchione, C., and Castellini, W. Androgens in breast cancer. II. Endometrial adenocarcinoma and breast cancer in married postmenopausal women. *Am. J. Obstet. Gynecol.*, 118: 173-178, 1974.
- Grattarola, R., Secreto, G., and Recchione, C. Androgens in breast cancer. III. Breast cancer recurrences years after mastectomy and increased androgenic activity. *Am. J. Obstet. Gynecol.*, 121: 169-172, 1975.
- Greenwood, F. C., Hunter, W. M., and Glover, J. S. The preparation of  $^{131}\text{I}$ -labeled growth hormone of high specific radioactivity. *Biochem. J.*, 89: 114-118, 1963.
- Horn, H., and Gordan, G. S. Plasma testosterone in advanced breast cancer. *Oncology (Basel)*, 30: 147-151, 1974.
- Ismail, A. A., Niswender, G. D., and Midgley, A. R., Jr. Radioimmunoassay of testosterone without chromatography. *J. Clin. Endocrinol.*, 34: 177-184, 1972.
- Kodama, M., Kodama, T., Minra, S., and Yoshida, M. Hormonal status of breast cancer. III. Further analysis of ovarian adrenal dysfunction. *J. Natl. Cancer Inst.*, 59: 49-54, 1977.
- Malarkey, W. B., Schroeder, L. L., Stevens, V. C., James, A. G., and Lanese, R. R. Twenty-four-hour preoperative endocrine profiles in women with benign and malignant breast disease. *Cancer Res.*, 37: 4655-4659, 1977.
- McFadyen, I. J., Prescott, R. J., Groom, G. V., Forrest, A. P. M., Golder, M. P., Fahmy, D. R., and Griffiths, K. Circulating hormone concentrations in women with breast cancer. *Lancet*, 1: 1100-1102, 1976.
- Midgley, A. R., Jr. Radioimmunoassay: A method for human chorionic gonadotropin and human luteinizing hormone. *Endocrinology*, 79: 10-18, 1966.
- Moore, J. W., Clark, G. M. G., Bulbrook, R. D., Hayward, J. L., Murai, J. T., Hammond, G. L., and Siiteri, P. K. Serum concentrations of total and non-protein-bound oestradiol in patients with breast cancer and in normal controls. *Int. J. Cancer*, 29: 17-21, 1982.
- Page, D. L., Vander Zwaag, R., Rogers, L. W., Williams, L. T., Walker, W. E., and Hartmann, W. H. Relation between component parts of fibrocystic disease complex and breast cancer. *J. Natl. Cancer Inst.*, 61: 1055-1063, 1978.
- Rosner, W. A simplified method for the quantitative determination of testosterone-estradiol-binding globulin activity in human plasma. *J. Clin. Endocrinol. Metab.*, 34: 983-988, 1972.
- Secreto, G., Fariselli, G., Bandieramonte, G., Recchione, C., Dati, V., and Di Pietro, S. Androgen excretion in women with a family history of breast cancer or with epithelial hyperplasia or cancer of the breast. *Eur. J. Cancer Clin. Oncol.*, 19: 5-10, 1983.
- Secreto, G., Recchione, C., Cavalleri, A., Miraglia, M., and Dati, V. Circulating levels of testosterone,  $17\beta$ -oestradiol, luteinising hormone, and prolactin in postmenopausal breast cancer patients. *Br. J. Cancer*, 47: 269-275, 1983.
- Sherman, B. M., and Korenman, S. G. Hormonal characteristics of the human menstrual cycle throughout reproductive life. *J. Clin. Invest.*, 55: 699-706, 1975.
- Sheth, N. A., Ranadive, K. J., Suraiya, J. N., and Sheth, A. R. Circulating levels of prolactin in human breast cancer. *Br. J. Cancer*, 32: 160-167, 1975.
- Siiteri, P. K., Hammond, G. L., and Nisker, J. A. Increased availability of serum estrogens in breast cancer: a new hypothesis. In: M. C. Pike, P. K. Siiteri, and C. W. Welsch (eds.), *Branbury Report 8: Hormones and Breast Cancer*, pp. 87-101. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory, 1981.
- Sinha, Y. N., Selby, F. W., Lewis, U. J., and Vanderlaan, W. P. A homologous radioimmunoassay for human prolactin. *J. Clin. Endocrinol. Metab.*, 36: 509-516, 1973.
- Sitruk-Ware, L. R., Sterkers, N., Mowszowicz, I., and Mauvais-Jarvis, P. Inadequate corpus luteal function in women with benign breast diseases. *J. Clin. Endocrinol. Metab.*, 44: 771-774, 1977.
- Swain, M. C., Bulbrook, R. D., and Hayward, J. L.: Ovulatory failure in a normal population and in patients with breast cancer. *J. Obstet. Gynaecol. Br. Commonw.*, 81: 640-643, 1974.
- Zumoff, B., Levin, J., and Kream, J. Subnormal 24-hour mean concentration and abnormal 24-hour profile of plasma LH in the luteal phase in women with breast cancer. *Anticancer Res.*, 1: 305-308, 1981.