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STUDIES ON URINARY ESTROGENS IN PATIENTS WITH ADVANCED BREAST CANCER IN REFERENCE TO OOPHORECTOMY AND ADRENALECTOMY*

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Urinary three estrogens (estrone, estradiol-17 β and estriol) in patients with advanced breast cancer before and after oophorectomy and adrenalectomy were chemically determined in an attempt to evaluate response of cancer to the surgery and its subsequent relapse. Results are as follows:

1. The existence of adrenal estrogens was evidenced indirectly in control subjects.

2. The high levels of preoperative urinary estrogens, probably due to the adrenal gland, were observed more frequently in aged patients with advanced breast cancer than in aged normal women. Most of the patients who had shown urinary hyperestrogenism in the basal and reserved estrogen levels obtained remission by the surgery.

3. The levels of postoperative persistent urinary estrogens in patients with advanced breast cancer lowered during the withdrawal of maintenance corticoids, and did not rise after the administration of ACTH and/or gonadotrophin. Any significant difference between the postoperative urinary estrogen levels in remission and relapse groups was not demonstrated. It may be therefore suggested that postoperative urinary estrogens are mainly derived from maintenance corticoids, and that cancer relapse after initial response to oophorectomy and adrenalectomy is not due to the re-increase of urinary estrogens based on the functioning of the accessory adrenocortical tissue.

INTRODUCTION

It is generally accepted that estrogens are closely related to the growth of some breast cancers, whereas estrogens are produced by the adrenal cortex as well as by the ovary.

In view of endocrine ablation therapy, oophorectomy for the patient with advanced breast cancer was first tried by Beatson (1896)[6] to result in regression of the neoplasm. It was suggested that this regression was induced by the elimination of the principal source of estrogens.[53] It was however reported that some cancers regressed by oophorectomy recur in relation to the re-

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increase of urinary estrogens.[50] Under such circumstances, Huggins and Bergenstal (1951) considered the adrenal cortex to be a source of extra-ovarian estrogens and they proposed adrenalectomy in addition to oophorectomy for patients with advanced breast cancer.[32,33]

In our department, since 1956, about 70 patients with advanced breast cancer have been submitted to this surgery or the adrenal-portalisation to inactivate estrogens in the liver. As reported by Nagai (1960), about a half of them have had clinical improvement.[43]

The purpose of this paper is to describe some results of investigations on urinary estrogens in advanced breast cancer patients before and after oophorectomy and adrenalectomy.

The results of the chemical determination of urinary estrogens before the surgery in breast cancer patients were compared with those in control subjects and studied from aspects of age of subjects and the clinical effect of surgery to find out some indications for oophorectomy and adrenalectomy.

The results after the surgery in breast cancer patients were compared with those before surgery and they were studied in views of the clinical effect of surgery, the relapse of cancer and the factors influencing urinary estrogen levels to find out possible causes of persistent estrogens and of the cancer relapse.

Urinary three estrogens (estrone, estradiol-17 β and estriol) were studied with the concept of the excretion of basal and reserved estrogens. The basal estrogen excretion was estimated on the urine collected in an early half period of the follicular phase in the menstrual cycle. The reserved estrogen excretion was also studied by administering gonadotrophin.

In this paper, the indication for oophorectomy and adrenalectomy is discussed in connection with the reserved estrogens.

CLINICAL MATERIALS AND METHODS

Clinical Materials

Forty patients with advanced breast cancer and 47 control subjects were submitted to estrogen measurements. Of all the control subjects, 23 healthy women were employed to compare with the breast cancer patients, and 2 women with amenorrhea due to ovarian insufficiency, 9 oophorectomized women, 9 subjects with Cushing's syndrome and 4 women with adrenogenital syndrome were employed for the research of the ovarian and adrenal estrogens. All the subjects involved in this study were women except 4 men with Cushing's syndrome.

Of 40 cases with advanced breast cancer, ranging in age from 30 to 82 years, 21 cases were submitted to oophorectomy and adrenalectomy. Thirteen cases out of the 21 obtained subjective and objective improvement assessed

at one month after the surgery, and in the remaining 8 cases no effect was observed.

Chemical Determination of Urinary Estrogens

Estimations were performed on at least two complete 48-hour collections of urine from each subject. Urinary estrogens were chemically determined by completing five procedures of hydrolysis, extraction, purification, separation and fluorometry. The hydrolysis, extraction and purification for phenolic substances were carried out by the method of Engel[20] and of Napp and Kersten.[46] The separation of phenolic substances to estrone, estradiol-17 β and estriol was done by a modification of the method of Hosoi and Kanbegawa.[31]

Urine (50–200 ml) was hydrolyzed by boiling with conc. HCl (15 vol.%) for 30 min., cooled rapidly and extracted three times with peroxide free ethyl ether (50 vol.%). The ether extract was washed first with saturated $NaHCO_3$ solution (20 vol.%) and then with water (10 vol.%). The ether was evaporated and the residue I was dissolved in toluene (20 ml). The toluene solution was then extracted twice with 1 N-NaOH (30 ml). The NaOH layer was adjusted to pH 9 with HCl and re-extracted four times with ether (25 vol.%). The ether layer was washed with water (10 vol.%) and then evaporated to dryness. The dry residue II was dissolved in thiophene free benzene (1 ml), and estrogens were separated by the column chromatography.

The alumina column (Al_2O_3 2.7 g, Merck Brockman No. II) employed here, was supported by cotton at the bottom of a chromatogragh tube of 6 mm internal diameter, 40 ml capacity.

After the benzene solution was adsorbed, the container was rinsed with 1 ml of 1% methanolbenzene (1% MB) to avoid loss of sample material. The rinsing was adsorbed also, and then the first eluent, 23 ml of 1% MB, was introduced into the column. The rate of adsorption of the benzene solution of residue II was performed slowly by natural dropping, but the rate of flow of the eluents (1% MB 24 ml, 5% MB 14 ml, 30% MB 20 ml) was adjusted to approximately 0.8 ml/min. by applying slight pressure.

The chromatograghic separation of a mixture of estrone, estradiol-17 β and estriol was shown in Fig. 1. Estrone was eluted with 1% MB, estradiol-17 β with 5%MB, and estriol with 30%MB.

Each fraction was evaporated to dryness. Each dry residue III was heated after addition of 70% sulphuric acid (5 ml) at 80°C for 30 min. The fluorescence appeared by this procedure must be derived from estrogens and some contaminated urochromogens. To remove the fluorescence derived from estrogen, 1% hydrogen peroxide (2 drops, less than 0.1 ml) was added to the fluorescent solution and after mixing well, the mixture was heated at 80°C for 30 min. Since the remaining fluorescence was chiefly due to the con-

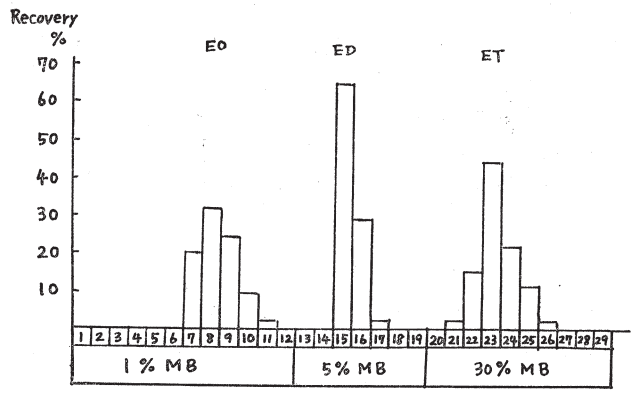

FIG. 1. Chromatographic separation of a mixture of 40 μg estrone (EO), 40 μg estradiol-17 β (ED) and 60 μg estriol (ET), using alumina column of 6 mm diameter.

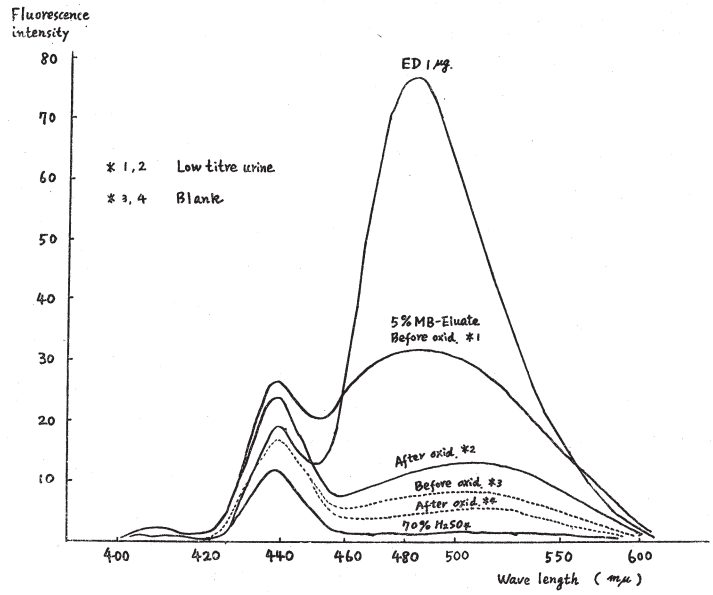

FIG. 2. Fluorescence spectra of the estrogen and urochromogen obtained from the urine of an oophorectomized woman.

The fluorescence of the eluate of 5% methanolbenzene before oxidation must be owing to the presence of estradiol and urochromogen, while that after oxidation is not related to estradiol.

taminant, the amount of each estrogen included in the urine was calculated from the difference between the two readings of the fluorescence intensity of each eluate, before and after oxidation, on Yagi's photoelectric fluorometer. Filters used in this experiment were UV-V_2, UV-O_2 and FL-480. They had a

excitation at 436 mμ and a peak transmission at 480 mμ. The fluorescence of each estrogen had a peak at 480–487 mμ, while that of urochromogen has a peak at somewhat higher wave-length than the above, as shown in Fig. 2.

The standard calibration curve was linear within the range of 0.01–1 μg for estrone and estradiol and of 0.02–2 μg for estriol. It was therefore needed that urine specimen should include each estrogen within the above range. The more the amount of each estrogen increased, the greater the quenching occurred.

Biological Determination of Urinary Estrogens

This procedure was performed to detect the biological activity of minute amount of phenolic substances in urine after oophorectomy and adrenalectomy, by using the method of Kobayashi and Nakayama.[35]

The dry residue II descrived above was dissolved in propylene glycol. The solution (0.05 ml) was poured into the vagina of spayed rats. This procedure was repeated once again after 24 hours. Vaginal smears were examined microscopically before initial pouring, and at the periods of 48, 60 and 72 hours after. The biological activity in urine specimen was assessed by grade of the cornification of vaginal epithelial cells.

Determination of Reserved Urinary Estrogens Responding to Gonadotrophin

"The reserved urinary estrogens" was named against the basal urinary estrogens. The level of reserved urinary estrogens was indicated as the increased amount over the basal level of estrogens per 24 hours (or a day) obtained from 48-hour collection of urine following the first administration of pregnant mare serum gonadotrophin (1 000 I.U./day i.m. ×2).

This method of administering gonadotrophin has the following characters, as partially reported in women without endocrine diseases.[70,71] (1) On the activity to increase urinary total estrogen, gonadotrophin was stronger than ACTH (Table 1). (2) The rate of increase of either estradiol (90%) or

TABLE 1. Comparison of Effects of ACTH and Gonadotrophin on Urinary Hormone Levels

	ACTH*1		Gonadotrophin*2	
	Number of cases	Increase of hormones	Number of cases	Increase of hormones
17-Hydroxy corticosteroids*3	69	8.9(mg/day)	22	1.4(mg/day)
17-Ketosteroids*4	69	3.5(mg/day)	22	2.1(mg/day)
Total estrogen	9	4.8(μg/day)	18	7.2(μg/day)

*1 ACTH gel. 14 I.U./12 hrs. ×2, intramuscularly.
*2 Pregnant mare serum gonadotrophin 1 000 I.U./24 hrs., intramuscularly.
*3 Measured by the method of Reddy, Jenkins and Thorn.
*4 Measured by the method of Drekter.

estrone (70%) responding to gonadotrophin was generally higher than that of estriol (25%) on the first administration day. The mean values in 5 women are described in the bracket. (3) Twenty women out of 29, administered gonadotrophin (1 000 I.U./day ×2), showed the increase of urinary estrogen levels within the first two days after the surgery.

RESULTS

PREOPERATIVE URINARY ESTROGENS

Basal Urinary Estrogens

A. In control subjects:

In 47 control subjects with and without ovarian and adrenal diseases, the relations of basal urinary estrogens to the disease and age were investigated. Fig. 3 shows urinary total estrogen levels in control subjects. Results can be summarized as follows: (1) Two women with amenorrhea, due to ovarian insufficiency, showed extremely low levels of urinary total estrogen. (2) Most women without ovaries had the low levels within two months after oophorectomy, whereas two women out of four, long after oophorectomy, had the marked high levels. (3) In Cushing's syndrom, the highest level of total estrogen was observed in the urine of a 2-year-old boy, with a large adenoma of the left adrenal weighed 38 g. (4) In adrenogenital syndrome, the highest level of total estrogen was measured in the urine of a 29-year-old woman, with a huge adenoma of the right adrenal weighed 63 g. The level was decreased by removal of the adenoma from 160.7 μg/day to 26.9 μg/day. (5) In view of age in normal women, the mean level of urinary total estrogen was the highest in the 20–29 year-old group and showed gradual decrease with advancement of age. Over 60 and under 10 years old, the amounts of total estrogen were detected in the urine.

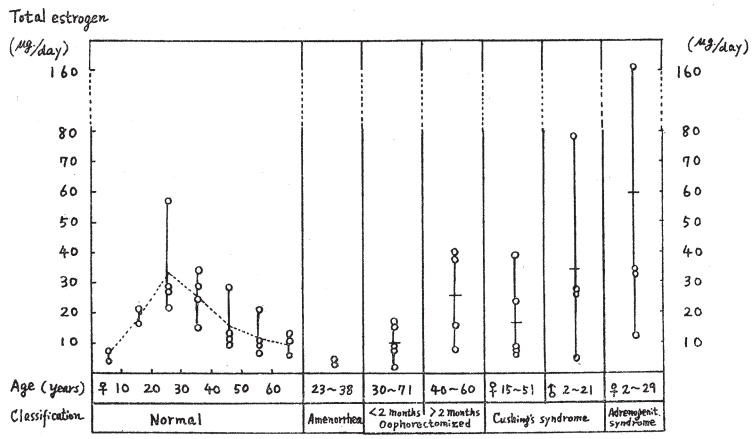

FIG. 3. Urinary total estrogen levels in control subjects.

TABLE 2. Basal Urinary Estrogen Levels in Control Subjects

		Age (years)	Number of cases	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total (μg/day)		EO+ED/Total (%)		ED/EO	
				Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Normal ♀		~9	2	1.4~1.4	1.4	0.6~ 1.4	1.0	1.9~ 3.7	2.8	3.9~ 6.5	5.2	43.0~51.3	46.2	0.4~1.0	0.7
		10~	2	3.0~3.1	3.0	3.5~ 3.8	3.6	9.4~13.7	11.6	16.2~20.3	18.2	32.6~42.0	37.3	1.1~1.3	1.2
		20~	4	4.7~8.8	6.1	5.8~10.0	7.4	11.0~40.0	19.6	21.5~56.6	33.1	29.2~57.6	40.8	0.9~1.8	1.3
		30~	4	2.3~6.5	4.4	4.1~11.4	7.5	8.6~19.0	13.6	15.0~33.8	25.2	42.7~54.0	46.0	1.1~3.4	1.9
		40~	4	1.8~5.4	2.7	2.2~ 6.7	3.5	5.2~16.4	9.1	9.2~28.5	15.3	33.3~43.5	40.5	1.1~1.6	1.3
		50~	4	0.7~4.6	2.3	1.0~ 4.5	2.7	5.0~12.0	6.9	6.7~21.1	11.9	25.3~46.8	42.1	0.9~1.9	1.3
		60~	3	1.0~2.4	1.8	1.3~ 2.6	1.8	3.1~ 8.9	5.8	5.4~12.7	9.4	29.9~46.1	38.2	0.6~1.3	1.0
Amenorrhea		23~38	2	0.3~1.2	0.7	0.2~ 0.8	0.5	1.4~ 3.0	2.2	2.4~ 4.4	3.4	31.8~45.8	38.8	0.2~2.6	1.4
Oophorect-omized	<2 Ms	30~71	5	0.2~2.7	1.2	0.3~ 4.8	2.4	1.0~10.9	6.5	1.5~16.5	10.1	21.5~47.5	33.6	0.8~3.0	1.8
	>2 Ms	40~60	4	1.4~8.8	4.8	1.9~ 9.2	5.6	4.4~22.0	14.1	7.7~40.0	25.5	42.9~45.0	44.1	0.6~1.4	1.0
Cushing's Syndrome	♀	15~51	5	1.2~8.8	3.1	0.7~13.7	5.8	3.7~16.0	7.8	6.2~38.5	16.7	30.7~58.4	47.8	0.6~3.2	1.8
	♂	2~21	4	1.1~9.3	5.8	1.4~22.9	10.1	2.3~46.0	18.3	4.8~78.2	34.2	41.2~58.9	50.2	0.9~2.5	1.7
Adrenogenital Syndrome		2~29	4	2.0~28.2	10.8	3.8~56.5	18.9	6.2~76.0	29.8	12.0~160.7	59.5	42.9~52.8	46.8	0.6~2.2	1.7

The results on each estrogen in control subjects are shown in Table 2. They generally resembled to the results on total estrogen shown in Fig. 3. Of all the normal subjects, the highest level of estradiol appeared in a 30-year-old normal women, but those of estrone and estriol were both observed in a 21-year-old woman. Comparing estrone with estradiol, estrone showed lower levels than estradiol in most cases. Exceptionally, 5 out of 23 normal women, 2 out of 9 oophorectomized women, 2 out of 9 cases with Cushing's syndrome and 1 out of 4 women with adrenogenital syndrome had higher levels in estrone than in estradiol. As shown in Table 2, the ratio of estradiol to estrone as well as the percentage of estrone-estradiol to total estrogen had the high mean value (over 1.7 and over 46%) in the 30–39 year-old normal group, in the Cushing's syndrome group and in the adrenogenital syndrome group. On the contrary, the ratio of estradiol to estrone was low (under about 1.0) in the under 10-year-old and the over 60-year-old group and in the over 2 months post-oophorectomized group. The percentage of estrone-estradiol was extremely low (33.6%) in the last group.

Concerning the mean percentage of each estrogen to total estrogen in each group, that of estrone was high (over 20%) in the under 10-year-old group, in the over 2 months postoophorectomized group and in the Cushing's syndrome male group. That of estradiol was high (over 28%) in the 30–39 year-old group, in the Cushing's syndrome group and in the adrenogenital syndrome group.

B. In breast cancer patients:

In 40 women with advanced breast cancer, ranging in age from 30 to 82 years, the relations of basal urinary estrogens to age and the clinical effect of surgery were investigated.

a. The relation to age

The results in breast cancer and control groups were compared in dif-

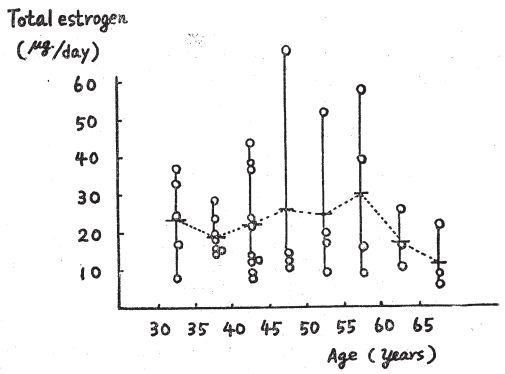

FIG. 4. Urinary total estrogen levels in breast cancer patients.

ferent ages. As shown in Fig. 4, there was some difference in the mean levels of urinary total estrogen as well as in their distribution type between the two groups.

The mean levels of total estrogen in breast cancer groups of over 40-year-old were always higher than those in normal groups. Above all, the highest mean level (30.3 μg/day) was found in the 55–59 year-old group. Since the age of 45 to 59 years known as climacteric showed irregular wide-ranged distribution of estrogen levels, special attention on such abnormal distribution was paid in this age group. Of 12 patients in this age group, 4 cases (1/3) had abnormally high levels of total estrogen, and the remaining 8 cases (2/3) had the levels within normal range.

The results on each estrogen in breast cancer patients are shown in Table 3. Of all the patients, the highest levels of estrone and of estriol appeared in a 49-year-old patient, whose total estrogen level was also the highest. The highest level of estradiol was found in another 54-year-old patient. In view of the age group, three groups of 45–59 year-old had higher mean levels of each estrogen than other groups. The highest mean levels of both estrone and estriol were observed in the 55–59 year-old group, while that of estradiol appeared in the 50–54 year-old group. Comparing these results with those in

TABLE 3-a. Basal Urinary Estrogen Levels in Breast Cancer Patients

Age (years)	Number of cases	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total(μg/day)		EO+ED/Total (%)		ED/EO	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean
30～34	5	1.4～ 8.7	4.2	1.5～10.2	6.8	2.7～19.0	12.5	7.5～32.8	23.5	40.2～64.0	48.7	0.5～4.8	2.3
35～39	7	2.0～ 6.5	3.8	0.4～ 8.7	4.0	8.6～15.1	11.3	14.4～28.1	19.1	26.3～54.1	39.1	0.1～1.8	1.1
40～44	10	1.4～ 7.5	3.2	1.9～13.4	5.2	4.2～25.6	13.6	7.7～43.9	22.0	27.0～54.8	38.8	1.1～2.7	1.6
45～49	4	0.8～11.7	4.2	2.7～12.5	5.4	6.4～43.9	17.0	10.4～68.1	26.3	35.6～38.8	37.3	1.1～4.0	2.0
50～54	4	1.5～ 9.7	4.3	2.9～13.9	7.0	5.0～27.9	13.1	9.4～51.5	24.4	42.7～52.1	46.9	1.4～2.0	1.8
55～59	4	1.3～ 9.1	4.6	1.2～11.8	6.8	6.4～39.6	18.9	8.9～57.4	30.3	28.1～51.1	38.4	1.0～2.0	1.4
60～64	3	2.1～ 4.6	3.3	2.6～ 7.5	4.7	5.5～13.4	9.4	10.2～25.5	17.4	44.0～47.4	45.9	1.2～1.6	1.4
65～82	3	1.0～ 3.6	2.3	1.3～ 4.0	2.6	3.1～14.0	7.0	5.4～12.7	8.9	29.9～55.7	42.9	0.6～1.3	1.0

TABLE 3-b. The Percentage of Each Estrogen to Total Estrogen in Breast Cancer Patients

Age (years)	Number of cases	EO (%)		ED (%)		ET (%)	
		Range	Mean	Range	Mean	Range	Mean
30～34	5	7.3～44.0	21.0	20.0～34.7	27.7	36.0～59.8	51.3
35～39	7	13.3～23.8	18.1	2.7～31.0	18.2	45.9～73.7	60.9
40～44	10	11.2～19.7	15.1	13.9～35.5	23.7	45.2～73.0	61.2
45～49	4	7.7～17.2	14.1	18.4～30.8	23.2	61.2～64.4	62.7
50～54	4	16.0～18.8	17.1	26.3～34.9	29.8	47.9～57.3	53.1
55～59	4	10.2～23.3	18.0	13.5～27.8	20.4	48.9～71.9	61.6
60～64	3	18.0～20.6	19.3	18.7～29.4	24.6	52.6～56.0	54.1
65～82	3	18.5～27.3	21.5	12.0～28.4	21.4	44.3～70.1	57.1

normal women, the mean levels of each estrogen in all the age groups of over 40 years old were always higher in breast cancer than in normal women.

Comparing estrone with estradiol, the levels of estrone were lower than those of estradiol in 35 patients, but in contrast, they were higher in only 5 patients. Both the ratio of estradiol to estrone and the percentage of estrone-estradiol to total estrogen were high (over 1.7 and over 46%) in the 30–34 year-old group as well as in the 50–54 year-old group.

The percentage of each estrogen to total estrogen was also investigated. That of estrone was high (over 20%) both in the 30–34 year-old group and in the 65–82 year-old group, and was low in the 45–49 year-old group. But that of estradiol was high (29.8%) in the 50–54 year-old group.

The results in breast cancer patients in general did not show such clear results as obtained from normal women. This fact would imply the existence of some abnormalities influencing the average. In this connection, 4 women of 45–59 year-old with abnormally high levels of total estrogen were specially investigated. The results are described as follows;

The mean levels (the mean percentages) of estrone, estradiol and estriol were 9.1 μg/day (17.4%), 12.3 μg/day (23.5%) and 32.6 μg/day (71.4%), respectively. These absolute levels are considerably high. The ratio of estradiol to estrone and the percentage of estrone-estradiol to total estrogen were 1.4 and 38.4%, respectively. These values are not high.

b. The relation to clinical effect of surgery

Twenty one patients submitted to oophorectomy and adrenalectomy were divided into two groups; the effective group (13 cases) and the non-effective group (8 cases), in an attempt to investigate the relationship between urinary estrogens and the clinical effect.

As presented in Fig. 5, the mean levels of both total estrogen and each estrogen had higher value in the effective group than in the non-effective group. The ratios of the mean level in the effective group to that in the non-effective group, were 28.0/14.9 in total estrogen, 5.4/2.9 in estrone and 15.8/8.5 in estriol. These results show striking differences

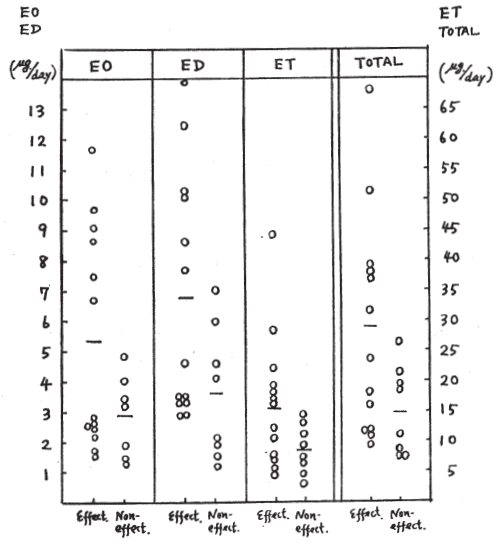

FIG. 5. Basal urinary estrogen levels in breast cancer patients with and without clinical effect of oophorectomy and adrenalectomy.

between the two groups (Table 6).

The mean percentage of estrone-estradiol to total estrogen was 43.2% (the range 31.0-54.4%) in the effective group, and 43.6% (28.1-64.1%) in the non-effective group. No significant difference between these percentages in the two groups was noticed.

The mean ratio of estradiol to estrone was 1.4 (1.0-1.9) in the effective group, and 1.2 (0.5-1.8) in the non-effective group. No significant difference between these ratios in the two groups was observed.

The ratios of estrone, estradiol and estriol to total estrogen were respectively 18.6% (11.1-25.7%), 24.7% (18.3-35.9%) and 56.7% (45.6-69.0%) in the effective group, and 21.0% (14.6-44.0%), 22.5% (13.5-32.1%) and 56.4% (36.0-71.9%) in the non-effective group. No significant difference between them was seen.

Reserved Urinary Estrogens

A. In control subjects:

Twenty four control subjects were divided into 4 groups, considering the sources of estrogens and were studied comparatively, as presented in Table 4.

The mean amount of reserved total estrogen increased by gonadotrophin was larger in the normal group than in the oophorectomized group. The largest increase in the latter group was observed in the urine of a 52-year-old woman, who had undergone oophorectomy about 5 years before. On the other hand, the mean increase in the Cushing's syndrome and the adrenogenital syndrome group was larger than that in the oophorectomized group. In the two women with Cushing's syndrome, the reserved urinary total estrogens were increased by removal of the adrenal glands.

Concerning each estrogen, the mean level of reserved estradiol was slightly higher than that of estrone in the three groups, excluding the adrenalectomized group. The mean level of estriol was always higher than that of estradiol

TABLE 4. Reserved Urinary Estrogen Levels in Control Subjects and Breast Cancer Patients

	Diseases		Number of cases	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total (μg/day)	
				Range	Mean	Range	Mean	Range	Mean	Range	Mean
Control	Normal		9	−0.6~3.5	1.0	−1.5~5.8	1.1	−1.6~7.0	2.0	−4.5~15.7	4.1
	Oophorectomized		6	−0.6~0.8	0.1	−0.5~1.8	0.4	−1.2~7.2	2.1	−1.8~ 9.4	2.6
	Cushing's or adrenogenital syndrome	Preoperative	6	−2.7~2.4	0.2	−0.1~3.4	0.7	−2.4~8.6	2.4	−5.2~13.6	3.3
		Adrenalectomized	3	−0.1~3.1	1.1	−0.7~1.4	0.4	1.0~5.4	3.8	1.7~ 9.8	5.3
	Breast cancer		20	−0.5~7.8	1.2	−0.8~5.7	1.6	−3.3~9.7	3.0	−2.9~20.5	5.8

in any group. The percentage of reserved estrone-estradiol to reserved total estrogen in urine was the highest (51%) in the normal group and the lowest (19%) in the oophorectomized group.

B. In breast cancer patients:

a. The relation to age

The level of reserved total estrogen as well as of each estrogen in breast cancer patients appeared on the average slightly higher than that in normal women. But no significant difference was observed between them. The percentage of reserved estrone-estradiol to reserved total estrogen did not show any difference between the two groups.

In view of age, 20 breast cancer patients were divided into 4 groups and were studied comparatively (Table 5). Of all the patients, the highest level of each estrogen appeared in the urine of a 54-year-old woman. The mean level of reserved each estrogen in the 50–59 year-old group was higher than

TABLE 5. Reserved Urinary Estrogen Levels in Breast Cancer Patients of Different Age-Groups

Age (years)	Number of cases	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total (μg/day)	
		Range	Mean	Range	Mean	Range	Mean	Range	Mean
30~39	9	−0.2~5.7	1.2	0.2~5.7	1.7	0.8~9.7	3.1	1.3~14.0	6.3
40~49	6	−0.5~1.2	0.2	−0.8~4.3	1.1	−3.3~8.0	1.6	−2.9~12.2	2.8
50~59	3	1.9~7.8	3.9	0.8~5.3	3.2	−1.6~7.4	3.4	3.9~20.5	10.6
60~	2	0.2~1.1	0.7	0.5~1.9	1.2	1.2~5.7	3.5	2.8~ 7.8	5.3

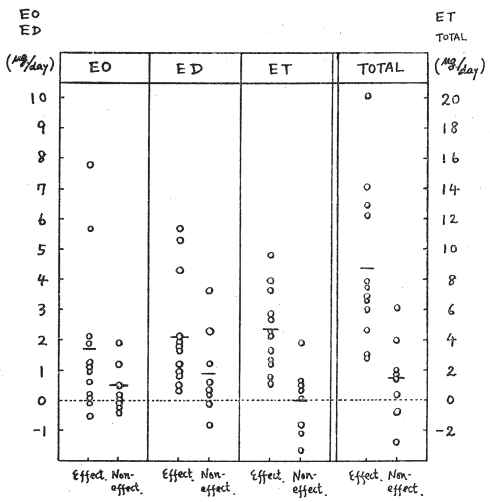

FIG. 6. Reserved urinary estrcgen levels in breast cancer patients with and without clinical effect of oophorectomy and adrenalectomy.

that in the 40–49 year-old group.

b. The relation to clinical effect of sugery

The relationship between reserved urinary estrogens and the effect of surgery is shown in Fig. 6. All the mean levels of reserved urinary estrogens in the effective group were higher than those in the non-effective group. Above all, the difference between the reserved estrone levels in the two groups was the smallest. According to the statistical estimation, the difference was most striking in total estrogen (Table 6).

In order to select the patients for oophorectomy and adrenalectomy, both levels of the basal and the reserved total estrogen were classified into three groups and named as shown in Table 7. Most of the patients in either hyper-

TABLE 6. Fiducial Limits of the Mean Urinary Estrogen Levels in Breast Cancer Patients

		EO (μg/day)	ED (μg/day)	ET (μg/day)	Total (μg/day)
Basal level	Effective	5.4±2.1	6.8±2.4	15.8±6.6	28.0±10.7
	Non-effective	2.9±1.1	3.5±1.9	8.5±3.3	14.9± 6.2
	Difference	+*1	−*2	+	+
Reserved level	Effective	1.7±1.6	2.1±1.3	4.9±1.0	8.7±4.0
	Non-effective	0.5±0.6	0.9±1.2	0 ±1.9	1.4±2.3
	Difference	−*3	+	+	+

*1 Significant difference (+) at $P<0.05$

*2 Significant difference (−) at $P<0.05$, but (+) at $P<0.06$

*3 Significant difierence (−) at $P<0.05$, but (+) at $P<0.20$

TABLE 7. Tried Classification of Urinary Estrogen Levels to Select the Patients for Oophorectomy and Adrenalectomy

Types of basal estrogenism	Basal excretion of total estrogen (μg/day)	Number of effective cases	Number of non-effective cases
Hyper-basal estrogenism	20≤	7	2
Medium-basal estrogenism	8~20	6	4
Hypo-basal estrogenism	<8	0	2

Types of reserved estrogenism	Reserved excretion of total estrogen (μg/day)	Number of effective cases	Number of non-effective cases
Hyper-reserved estrogenism	5≤	9	1
Medium-reserved estrogenism	2~5	3	1
Hypo-reserved estrogenism	<2	0	6

basal estrogenism (over 20 μg/day in the basal total) or hyper-reserved estrogenism (over 5 μg/day in the reserved total) showed good response to oophorectomy and adrenalectomy. On the contrary, any of the patients in either hypo-basal estrogenism (under 8 μg/day in the basal) or hypo-reserved estrogenism (under 2 μg/day in the reseved) obtained no benefit by surgery. In general, the higher the both basal and reserved estrogen levels in selected patients were, the greater the effectiveness of surgery became.

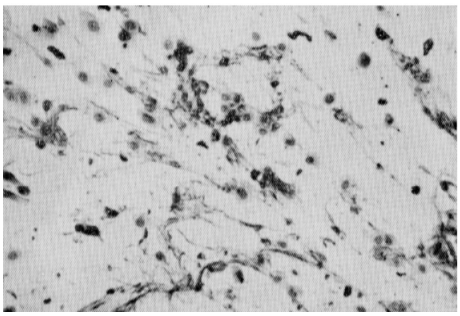

FIG. 7-a

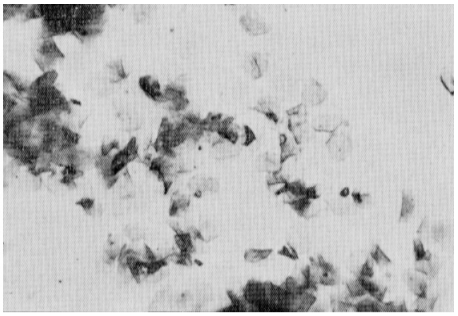

FIG. 7-b

FIG. 7-a. Microscopical pattern of no cornification of vaginal epithelial cells in a spayed rat.

FIG. 7-b. Microscopical pattern of cornification of vaginal epithelial cells in a spayed rat, showing the presence of estrogenic activity in the urine of an oophorectomized and adrenalectomized patient.

Postoperative Urinary Estrogens

Biological Evidence for Postoperative Urinary Estrogens

The existence of estrogenic activity in the urine of an oophorectomized and adrenalectomized patient was ascertained by the method of Kobayashi and Nakayama[35] (Table 8).

TABLE 8. Estrogenic Activity in the Urine of an Oophorectomized and Adrenalectomized Patient. Six Spayed Rats of 7 Submitted to the Method of Kobayashi and Nakayama, had Cornification of Their Vaginal Epithelial Cells 48 Hours after the Initial Administration of Phenolic Substances Obtained from 6-Hour Collection of Urine per Rat.

Hours after initial pouring	Sample	Control
48	6/7	0/6
60	3/7	0/6
72	0/7	0/6

Six spayed rats out of 7 submitted to this experiment, showed cornification of vaginal epithelial cells. According to the Kobayashi-Nakayama's standard, this means that an aliquot of urine obtained from the above patient had biological activity equivalent with over 1/1 000 μg estrone.

Comparison of Pre-and Post-Operative Urinary Estrogens

Chemically determined urinary estrogen levels before and after oophorectomy plus adrenalectomy were compared. The level of each estrogen was markedly decreased by the surgery. Although the amount of decrease was marked in estriol, estradiol and estrone, in this order, the rate of decrease was marked in estradiol and in estrone, comparing to that in estriol. Therefore the percentage of estrone-estradiol to total estrogen was significantly decreased by the surgery, from 43.3% to 28.0% (Table 9).

TABLE 9. Comparison of Pre-and Post-operative Urinary Estrogen Levels

	Number of cases	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total(μg/day)		EO+ED/Total (%)	
		Range	Mean	Range	Mcan	Range	Mean	Range	Mean	Range	Mean
Preoperative	21	1.3~11.7	4.5	1.2~13.7	5.6	2.7~43.9	13.2	7.5~68.1	23.3	28.1~64.3	43.3
Postoperative	21	0.3~ 2.8	1.2	0~ 2.5	1.1	1.5~13.6	5.9	2.5~17.7	8.2	12.0~56.9	28.0
Mean decrease		3.3		4.5		7.3		15.1			
The rate of mean decrease		73%		80%		55%		65%			

Monthly Levels of Postoperative Urinary Estrogens

It is well known that urinary estrogen levels decreased by oophorectomy increase again in the lapse of time. Fig. 8 answers to the inquiry whether such re-increase of urinary estrogen levels decreased by oophorectomy and adrenalectomy happens or not. Although the determination of estrogen levels

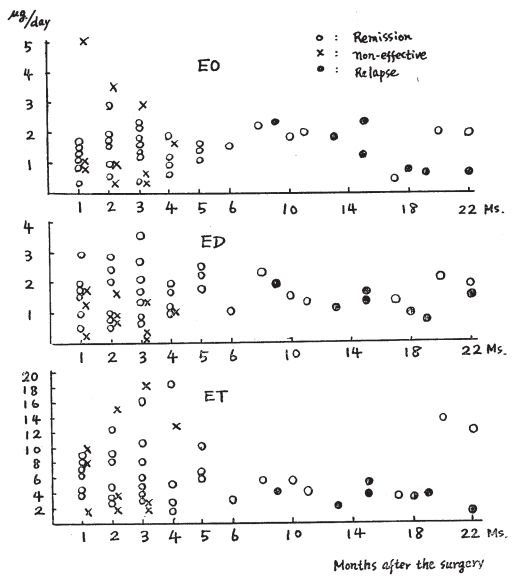

FIG. 8. Postoperative variation of urinary estrogen levels in breast cancer patients.

in a subject was performed several times a month, these values were averaged monthly and plotted as shown in this figure. Any tendency of increase of urinary estrogen levels within 22 months after the surgery was not observed.

Relation of Postoperative Urinary Estrogens to Remission and Relapse

The level of urinary each estrogen after the surgery appeared slightly higher in the effective group than in the non-effective group, but no significant difference between the levels in the two groups was observed (Table 10).

Since the response of cancer to the surgery is always followed by relapse, the effective group was divided into the two groups in the stage of remission and relapse to study them comparatively. All the levels of estrone and estradiol in the relapse group were within the range of the levels in the remission group. However, those of estriol appeared slightly lower in the former group than those in the latter group.

TABLE 10. Postoperative Urinary Estrogen Levels in Breast Cancer Patients of Non-effective, Remission and Relapse Groups

Response of cancer	Number of determination	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total (μg/day)
		Range	Mean	Range	Mean	Range	Mean	Mean
Non-effective	10	0.3~5.2	1.7	0~1.7	0.9	1.3~18.1	7.5	10.1
Remission	33	0.2~2.9	1.4	0.5~3.6	1.7	1.3~18.4	6.9	10.0
Relapse	7	0.6~2.3	1.3	0.7~1.9	1.3	0.9~ 5.0	3.2	6.8

Relation of Postoperative Urinary Estrogens to Maintenance Corticoids

Oophorectomized and adrenalectomized patients were normally treated with a maintenance dose of cortisone (25 mg/day), cortisol (20 mg/day) or prednisolone (5–10 mg/day). When the administration of these corticoids were suspended, it was almost impossible for the patients to tolerate the corticoid withdrawal test for over 6 days. The levels of urinary estrogens during the tests were measured in 6 patients. As shown in Fig. 9, the marked decreases of estrone, estradiol and estriol were observed in the urine of all the patients. The mean rate of the decrease was 59% in estrone, 62% in estradiol and 64% in estriol. There was no significant difference between the rates in the remission and the relapse case.

In order to investigate the effect of various kinds of maintenance corticoids on the level of urinary estrogens, three corticoids were compared as shown in Table 11. The level of each estrogen was brought lower by maintenance with cortisol 20 mg/day than with cortisone 25 mg/day or with prednisolone 10 mg/day.

To investigate the effect of overdose of corticoid on the level of urinary estrogens, 25 mg/day cortisone and 50 mg/day cortisone were also compared. There was almost no significant difference.

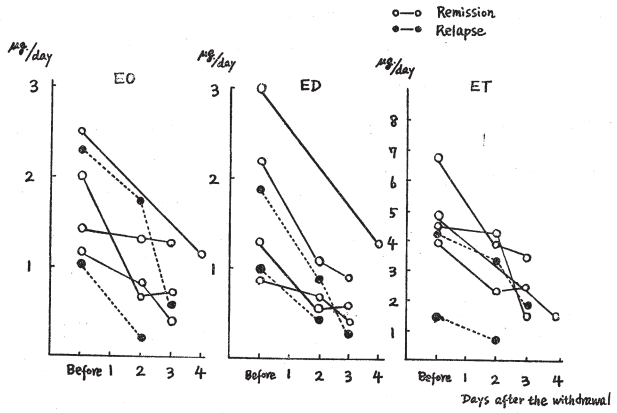

FIG. 9. Postoperative urinary estrogen levels in the withdrawal of maintenance corticoids.

TABLE 11. Postoperative Urinary Estrogen Levels in the Maintenance with Corticoids of Different Species and Dosages

	Corticoids	Number of determinations	EO (μg/day)		ED (μg/day)		ET (μg/day)		Total (μg/day)
			Range	Mean	Range	Mean	Range	Mean	Mean
Proper dosage	Cortisol 20 mg/day	9	0.3~1.5	1.0	0 ~4.1	1.1	2.2~12.0	4.0	6.1
	Prednisolone 10 mg/day	9	0.3~5.2	1.7	0.8~3.7	1.8	1.7~15.0	7.3	10.8
	Cortisone 25 mg/day	15	0.5~2.9	1.6	0.3~2.9	1.4	2.2~18.4	8.2	11.2
Overdosage	Cortisone 50 mg/day	4	0.4~2.4	1.7	1.3~2.1	1.8	3.3~16.2	9.5	13.0

Effect of ACTH and/or Gonadotrophin Administration on Post-Operative Urinary Estrogens

The effect of gonadotrophin administration (2 000 I.U./day) on postoperative urinary estrogen levels was investigated three times in two patients 10 to 17 months after the surgery. No significant increase was observed in this experiment (Table 12).

On the other hand, the 2-day administration of ACTH (40 I.U./day) and the subsequent 2-day administration of ACTH (40 I.U./day) plus gonadotrophin (2 000 I.U./day) to a patient showed no increase of urinary estrogen levels.

Effect of Testosterone or Estradiol Administration on Post-Operative Urinary Estrogens (Table 13)

Urinary estrogen levels were increased by the intramuscular injection of testosterone propionate (250 mg/day) to two patients long after the surgery. Above all, the rate of increase of estrone-estradiol appeared slightly greater than that of estriol.

TABLE 12. Effects of ACTH and/or Gonadotrophin on Postoperative Urinary Estrogen Levels

Method of administration	Case number	Months after surgery	Before and after administration	EO (μg/day)	ED (μg/day)	FT (μg/day)	Total (μg/day)
GTH 2 000 I.U./day×1	25	10	Before	2.4	0.8	4.1	7.3
			After*1	1.8	1.3	4.8	7.9
	35	17	Before	0.2	1.0	3.6	4.8
			After	0.5	1.8	2.7	5.0
	26	12	Before	1.5	2.5	2.0	6.0
			After	2.2	2.0	2.2	6.4
ACTH 40 I.U./day×2*2	51	2	Before	1.8	2.3	6.8	10.9
			1 day after	1.5	1.9	5.1	8.5
			2 days after	1.3	1.8	4.2	7.3
GTH 2 000 I.U./day and ACTH 40 I.U./day×2*			3 days after	1.0	1.2	3.2	5.4
			4 days after	1.4	1.2	3.0	5.6

*1 On the administration day
*2 Administered on the 1st and 2nd days
*3 Administered on the 3rd and 4th days

TABLE 13. Effects of Testosterone and Estradiol on Postoperative Urinary Estrogen Levels

	Case number	Months after surgery	Before and after administration	EO (μg/day)	ED (μg/day)	ET (μg/day)	Total (μg/day)
Testosterone propionate 250 mg/day	25	18	Before	0.7	1.2	2.4	4.3
			After*1	1.2	2.5	2.8	6.5
	29	15	Before	1.2	1.5	5.0	7.7
			After	3.2	2.2	6.8	12.2
Estradiol valerianate 10 mg/day	25	6	Before	1.5	0.3	3.5	5.3
			After*2	4.2	5.2	12.3	21.7
	30	4	Before	1.6	1.0	12.8	15.4
			After	5.4	5.7	19.3	30.4

*1 On the administration day of testosterone propionate (mg/day)
*2 On the 3rd administration day of estradiol valerianate (mg/day×3)

On the other hand, urinary estrogen levels were markedly increased to almost preoperative normal levels by the intramuscular injection of estradiol valerianate (10 mg/day×3). The percentage of each estrogen to total estrogen following the administration of estradiol was close to that of preoperative urinary each estrogen.

DISCUSSION

Classical three estrogens (estrone, estradiol-17 β and estriol) in the urine of breast cancer women were measured in this study. Since 1953, many other

new estrogens have been isolated from human urine.[36)37)] They are all derivatives of classical three estrogens, having characters: (1) An aromatic Ring A (2) Oxygen substituent at C-17 (3) A phenolic OH group at C-3.[27)] Once they were called "impeded estrogens",[47)] because they were in general less potent than active estrogens such as estrone and estradiol in biological activities. However it is said that classical three estrogens represent only one quarter of all the estrogens excreted.[40)42)] The effect of new estrogens on the growth of breast cancer has hardly been demonstrated, since there is no suitable clinical method for the determination of new estrogens. With the same reason, only three but main estrogens were measured in this study.

Sources of Urinary Estrogens

As sources of urinary estrogens, the ovary, the adrenal gland, the placenta and the testicle have been known. In this respect, the ovary is most significant in normal menstruating young women, while the adrenal gland has greater part in postmenopausal and in adrenocortical-hyperfunctioning women, as indicated previously in this paper.

There have been some evidences that the adrenal gland participates in the biosynthesis of estrogens; (1) Estrone was chemically isolated from the bovine adrenals (Beall, 1939).[5)] (2) The experimental oophorectomy in certain strains of mice was prone to develop nodular cortical hyperplasia or adrenal cortical carcinomata, increasing estrogenic activities (Fekete, 1941).[21)] (3) The clinical extirpation of femininising adrenocortical tumors improved secondary sexual signs (Frank, 1934).[23)] (4) Embriologically the adrenal cortex and the gonadal medulla are derived from adjacent areas of the urogenital ridge (Witschi, 1950).[74)]

Paying attention to the fact that adrenal glands produce estrogens, urinary estrogens were investigated in views age and disease. In view of age, urinary estrogens of normal women showed low levels at the age of 5 years and steep ascent with the advancement of age to reach the maximum level at 20-29 years old, and then gradual descent by almost over 80 years old.

Talbot (1956)[66)] reported that a trace of urinary estrogen was already detected at 3 years old and the increase became marked in the age over 11 years. According to Nakayama (1959),[45)] urinary estrogens were in lower levels by 8 years of age, began a gradual increase at 9 years old, and then showed a marked increase following menarch. Albright (1947)[2)] mentioned that urinary estrogen levels began to rise suddenly in puberty and to fall suddenly following menopause, whereas adrenopause occurred later than menopause. According to the Tauchi's study (1961)[68)] in autopsy cases, the adrenal weight showed almost no decrease in the aged. In the adrenal cortex, the zona reticularis closely related to estrogens was thicker than other zones during the age of 20 to 60 years (Rotter, 1949).[55)69)] Sakurai (1959)[56)] reported

that urinary estrogen levels rose again in certain stage following menopause. It is therefore suggested that the adrenal gland plays a greater role around and after menopause than at younger age, regarding biosynthesis of estrogens in normal women.

In this study, urinary estrogens were also much excreted in some women with oophorectomy and with adrenocortical hyperfunction. The re-increase of urinary estrogens following oophorectomy in women had been reported by Dao (1953),[18] Struthers (1956),[63] West (1958)[73] and Sengoku (1959).[58] According to Hadfield (1956),[28] it may be due to the increase of gonadorophin secretion. Nishikawa (1955)[49] observed maximum levels in gonadotrophin secretion at the age of 60–70 years. On the other hand, the adrenocortical hyperplasia following oophorectomy in mice had been found by Wooley (1939),[75] Fekete (1941)[21] and Flux (1954).[22]

The high level of urinary estrogens in Cushing's or adrenogenital syndrome was, usually in this study, accompanied with that of 17-ketosteroids or 17-hydroxycorticosteroids, and in certain cases it fell down to low level.

From these data, it is recognized that the adrenal gland takes part in the biosynthesis of estrogens in certain women with oophorectomy and with Cushing's or adrenogenital syndrome, as well as in normal women.

It was shown in this paper that the urinary reserved estrogens which responded to pregnant mare serum gonadotrophin were derived from the ovary and the adrenal gland in certain women with the high basal level of urinary estrogens. Pesonen (1960)[51] reported that in women with adrenal hyperplasia, urinary estrogen levels rose in response to gonadotrophin. It has been suggested by many investigators that in normal women ovarian estrogens increased by gonadotrophin stimulate the secretion of adrenal estrogens, and that gonadotrophin stimulates directly adrenal estrogens without mediation of the ovary. The direct influence of chorionic gonadotrophin on the adrenal gland in oophorectomized mice was indicated by Botella-Llusia (1950)[9] and that of pregnant mare serum gonadotrophin in hypophysectomized rats was also demonstrated by Akasu (1959).[1] Akasu concluded that in oophorectomized women, the effect of pregnant mare serum gonadotrophin as FSH like substance on stimulating the secretion of adrenal estrogens was stronger than that of chorionic gonadotrophin as LH like substance.

Characters of Urinary Estrogens in Breast Cancer Patients

Urinary estrogen levels in breast cancer patients have been compared with those in normal women by many investigators. According to Smith (1954),[61] Brown (1958)[11] and Sengoku (1959)[58], they were higher in patients than in normals. However, Ross and Dorfman (1941),[54] Taylor and Twomby (1943)[67] and Masuda (1957)[41] reported that the levels in patients were normal or lower. Most of authors who repoted normal or lower estrogen levels obtained the

results that the ratio of estrogen to androgen or progesterone was higher in patients with breast cancer.[25) 41)]

Although no striking difference between urinary estrogens in breast cancer and normal groups was demonstrated in this study, there was found small difference when every age group was compared. The mean level of urinary estrogens was markedly higher in the patients than in normals during the age of 45 to 59 years. Of all the patients included in this age group, one third of patients had the adnormally high levels and the remaining two third were within normal levels. On the other hand, one seventh of normal women had the relatively high level. As mentioned before, the adrenal gland seems to be closely related to the high level of urinary estrogens. According to Nagai (1960),[43)] most of the adrenal glands removed from breast cancer patients had histological abnormalities. This result agrees with the finding in breast cancer mice reported by Masuda (1957).[41)] Nagai and Nishi (1962)[44)] found that the adrenal weight and the thickness of the inner layer consisted of zona fasciculata and zona reticularis were both correlated to the urinary total estrogen level measured by the author.

Regarding each estrogen, the percentage of estrone-estradiol to total estrogen at 50-59 years of age in breast cancer patients was as high as at 30-39 years of age in normal women. Especially the percentage of estradiol to total estrogen in this age group was generally as high as that in adrenal hyperfunction. The rate of increase of the urinary total estrogen level in breast cancer patients was higher at 50-59 years old than at 40-49 years old.

It is concluded that the hyperestrogenism in the basal as well as in the reserved estrogen excretion may reflect certain abnormalities of the adrenal gland and the ovary as sources of estrogens.

What caused such adnormalities in the ovarioadrenal system of breast cancer patients? In view of the personal history on sexual functions, the non-partus, the abortus, the irregular menstrution and the abnormal lactation were observed more in the patients than in normals. Above all, non-partus women occupied about 30% of all the patients with advanced breast cancer. According to Fujimori (1960),[25)] these abnormalities in postmenopausal patients with breast cancer were observed more than those in the premenopausal patients. These abnormalities in sexual functions may induce abnormalities in the morphology and the function of the ovario-adrenal system.

Significance of Urinary Estrogens on Surgical Indications

According to Birke (1958),[8)] McAllister (1960)[42)] and Bulbrook (1960),[16)] there was no difference between the preoperative urinary estrogens in the effective group and in the non-effective group.

Huggins (1954),[34)] Dao (1957),[18)] Sengoku (1959)[58)] and Nagai (1960),[43)] however, reported that the mean levels of urinary estrogens in the effective group

were higher than those in the non-effective group. The author's results on basal urinary estrogens showed the same difference as reported by Huggins and others. Furthermore, the differences of the levels of reserved urinary total estrogen and estriol between the two groups were larger than those of estrone and estradiol.

The indications for oophorectomy and adrenalectomy were considered in views of the basal and the reserved total estrogen. As shown in Table 7, in hypo-basal estrogenism less than 8 μg/day, and in hypo-reserved estrogenism less than 2 μg/day, any effective case was not obtained. On the contrary, in hyper-basal estrogenism more than 20 μg/day and in hyper-reserved estrogenism more than 5 μg/day, the rate of effectiveness of the surgery was much greater.

Regarding the indications for surgery, it is better to recommend oophorectomy and adrenalectomy in the case of hyper-reserved hyper-basal estrogenism, because the growth of breast cancer depends greatly on both organs: the ovary and the adrenal. On the other hand, there is no indication for this surgery in the case of hypo-reserved hypo-basal estrogenism.

In medium-reserved or medium-basal estrogenism, it is necessary to investigate other factors, such as 17-ketosteroids, 17-hydroxy corticosreroids in urine, other clinical features of the cancer growth and so forth. Yoshida (1961)[78] reported that this surgery was less effective in the most cases of hypofunction of the adrenal cortex. According to Allen (1957),[3] in the patients ultimately improving after the surgery, the ratio of 11-deoxy to 11-oxy (or 11-hydroxy) 17-oxosteroids was over 1, and that in failures was under 1. Bulbrook and Greenwood (1960)[16] mentioned that, in a formula: 80−80 (17-OHCS (mg/day))+aetiocholanolone (μg/day), positive values were more likely to be associated with a successful response and negative ones with no benefit. Sengoku (1959)[58] indicated that the ratio of estrogen to the 4th and 5th fractions of 17-ketosteroids in the effective group was greater than that in the non-effective group.

On the other hand, most of the breast cancers of slow growth resulted in success of the surgery, as also reported by Huggins (1954)[34] and Nagai (1960).[43]

Thus, the effectiveness of oophorectomy and adrenalectomy may be expected in the following advanced breast cancers. (1) Breast cancer in case of urinary hyper-reserved hyper-basal estrogenism, or urinary relative hyperestrogenism to androgens and other hormones. (2) Breast cancer regressed by oophorectomy, or aggravated by estrogen administration. (3) Breast cancer growing slowly in postmenopausal women.

Sources of Postoperative Urinary Estrogens

Persistent urinary estrogens in oophorectomized and adrenalectomized patients have been chemically measured by Strong *et al.* (1956),[62] Bulbrook

and Greenwood (1957),[12] Hellström (1958),[29] Diczfalusy (1958),[19] Birke (1958),[8] Hobkirk (1959),[30] Takahashi (1959),[65] Sengoku (1959),[58] Nagai (1960),[43] Mc Allister (1960),[42] Sim (1961),[60] Chang (1961)[17] and so forth. The existence of persistent urinary estrogens were also evidenced biologically by Bulbrook, Greenwood and Williams (1957).[13,14,15] There has been however some discrepancy between the chemical and biological determinations.

The postoperative data presented in this paper proved, chemically and biologically, the persistence of urinary estrogens.

On the sources of persistent urinary estrogens, the following four factors are considered; (1) Retention of preoperative estrogens in the body (2) Intake of estrogen with diet (3) Secretion of estrogens by accessory adrenals or parovaries (4) Conversion of maintenance corticoids into estrogens.

The retention of estrogens in the body fat was raised by Crook.[40] If such a retention is a main source of persistent estrogens, postoperative urinary estrogen levels should fall down gradually with months after the surgery. In the author's results, the levels of urinary estrogens fell down rapidly by the surgery, thereafter no significant change of the levels was observed for 22 months. Therefore, it is hard to believe the retention of estrogens as a main source of persistent estrogens.

According to the report of Briggs (1957),[10] the daily intake of estrogens in meat by one person was less than 1 μg, even if the meat was obtained from the animal which had been administered estrogen before slaughter. The author experienced that persistent urinary estrogen levels were not so influence by any sort of daily diet, if patients avoided taking vegetable laxatives or other drugs during the period of examination.[14]

Secretion of estrogens by accessory adrenals was emphasized by Symington (1957),[64] Bulbrook (1957)[12] and Shibuzawa (1957).[59] But it was not so postulated by Hobkirk (1957)[30] and Sim (1961).[60] Sim mentioned as follows: (1) 17-Ketosteroids and 11-oxygenated fraction derived from maintenance cortisone fell to low levels during cortisone deprivation, while 11-deoxy-17-ketosteroids, which are exclusively of endogenous adrenocortical origin, were consistently absent from the urine whether cortisone was taken or not. (2) 17-Hydroxycorticosteroids were absent from the peripheral blood on the 3rd day of cortisone withdrawal. (3) An infusion of corticotrophin did not stimulate their secretion.

Similar results to the above were obtained on 17-ketosteroid fractions in urine by Yamaoka, (1961)[77] and on 17-hydroxycorticosteroids in blood by Furukawa (1962).[26] No increase of urinary estrogens was observed by the administration not only of ACTH but also of gonadotrophin or ACTH plus gonadotrophin, as described previously in this paper. No increase of urinary 17-hydroxycorticosteroids and 17-ketosteroids following the administration of

ACTH and/or gonadotrophin was observed by Nagai and co-workers in a series of this study.[43,78]

On the other hand, by the withdrawal of cortisone or cortisol 17-hydroxycorticoids and 17-ketosteroids in urine fell to low levels, as reported by Nagai (1960)[43] and Yoshida (1961).[78] These decreases were also accompanied by the decrease of urinary estrogen as shown in this paper. In this withdrawal test, the increase of ACTH like activity in blood was observed by Fujikake (1961).[24,38] Sayers (1955)[57] reported that the high level of corticotrophin in adrenalectomized patients was reduced by treatment of cortisone. Furthermore, any adrenalectomized patient in this study could not clinically tolerate cortisone or cortisol lack for over 6 days. All the observations mentioned above does not support the hypothesis that the functioning of accessory adrenocortical tissue occurs after adrenalectomy and oophorectomy.

On the maintenance corticoids, two possibilities can be considered; one is a conversion of the corticoids into estrogens, the other is to mistake corticoid metabolites for estrogens.

The conversion of testosterone to estrogens, especially to estrone or estradiol, in castrated and adrenalectomized women was emphasized by West (1956).[72] Furthermore, Chang and Dao (1961)[17] evidenced the bioconversion of 4-C^{14}-cortisone acetate to 11 β-hydroxyestrone and 11 β-hydroxyestradiol in a castrated and adrenalectomized woman with breast cancer, but they could not evidence such after the administration of 4-C^{14}-cortisol.

In this study, testosterone administration to the postoperative patients resulted in the increase of urinary estrogens. This suggests the conversion of testosterone to estrogens.[4,76] Moreover, urinary estrogen levels during the maintenance with cortisone were prone to be higher than those during the cortisol maintenance. The decrease of urinary estrogens by corticoid withdrawal may support possibility of the conversion of corticoids, especially that of cortisone.

On the other hand, it must be taken in account that phenolic substances in the low titre urine may be partly contaminated by 17-oxosteroids and 17-ketogenic steroids.[15] When pure cortisone or cortisol was submitted to the method of estrogen determination employed here, any fluorescence equivalent to that of estrogen was not obtained. Furthermore, by the withdrawal of maintenance corticoids, urinary estrogen levels were decreased.

Therefore, postoperative persistent urinary estrogens may include some corticoid metabolites excluding corticoid itself.[14]

Consideration on Cancer Remission and Its Relapse

Although urinary estrogens did not be eliminated completely by oophorectomy and adrenalectomy, their levels resulted in the marked decrease of total estrogen and in the marked decrease of the percentage of estrone-estradiol

to total estrogen after the surgery.

Such decrease of the urinary total estrogen level in the remission group was more marked than that in the non-remission group. It may be therefore suggested that the striking fall of estrogen levels is one of the most significant cause of cancer remission.

Postoperative urinary estrogen levels showed no increase in the lapse of time. The relapse of cancer occured in such condition of no increase of urinary estrogen levels. Furthermore, postoperative urinary estrogen levels showed also no increase by the administration of ACTH and/or gonadotrophin.

Any significant correlation between postoperative urinary estrogens and cancer relapse was not demonstrated.

From these results, it may be assumed that the relapse of breast cancer is not due to the functioning of accessory adrenocortical tissue, but due to the proliferation of a few estrogen independent cells survived the condition of oophorectomy and adrenalectomy.

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