

# ENDOCRINOLOGICAL STUDIES ON NEOPLASTIC DISEASES OF THE BREAST IN THE LIGHT OF THE EXCRETION OF URINARY ESTROGENS

by

KEIGO NISHIYA

From the 2nd Surgical Division, Kyoto University Medical School

(Director : Prof. Dr. YASUMASA AOZAGI)

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## I. INTRODUCTION

The mammary gland must be considered a part of the female reproductive system, and as such is subject to the same stimuli throughout life as the other organs of reproduction. This is definitely illustrated during puberty and pregnancy, following the menopause. It is generally accepted at present that the breast, like the endometrium, is constantly undergoing periodic changes of progression and regression (hyperplasia and involution) during the normal menstrual cycle and is prepared each month for pregnancy. It is likely to have been established distinctly at present that these changes are mediated through the secretion of estrogens, progesterone and other hormones.

It is also apparent that the hormones may be of major importance in the genesis of diseases of the breast.

Recently, the number of patients with neoplastic diseases of the breast, especially, chronic cystic mastitis (mastopathia chronica) have markedly increased at our clinic and a relationship between chronic cystic mastitis and carcinoma of the breast, considering clinically, has again drawn the attention of the field. The disease called chronic cystic mastitis shows the complicated histological picture. Therefore, neoplastic, inflammatory and regressive theories have been histologically reported with regard to the concept of chronic cystic mastitis, consequently numerous names have been given to this disease. In 1927, Moszkowicz standardized biologically as

"mastopathia", considering this disease to be due to some dysfunction of the endocrine system. However, the interpretation for these endocrine dysfunction in chronic cystic mastitis and furthermore for the endocrinological relationship between this disease and breast cancer or other benign breast diseases still remains obscure at present.

For the purpose of investigating clinically these problems, the urinary excretion of estrogens and the ratio of urinary estrogens to 17-ketosteroids (determined by ISEDA, one of our coworkers) in patients with neoplastic diseases of the breast were observed.

For the estimation of estrogen in urine, the biological assay based upon the vaginal response on ovariectomized rats has been used by many investigators since the report of ALLEN and DOISY in 1923. However, this method presents the difficulties and uncertainties of the biological technic and necessitates the maintenance of a large animal colony and long time as well. On the other hand, because urinary excretion of estrogens in female is closely related to the ovarian activity during the menstrual cycle, so that the variation in excretion is present at different times in the monthly cycle, continuous daily observations for relatively long time seem to be clinically necessary. Such observations by the biological assay are considered to be very difficult.

Therefore, to begin with, the chemical determination of estrogen in urine by fluorometry was investigated and the evidence of its reliability for the clinical application was determined.

## II. METHODS OF RESEARCH

### 1. Subjects of the present study.

Among the patients with neoplastic diseases of the breast observed at our clinic during the past three years, 6 cases with fibroadenoma of the breast, 50 with chronic cystic mastitis and 53 with breast cancer diagnosed by the histological examination were selected as the subjects of the study, especially chronic cystic mastitis noted as precancerous condition was put at the focus of the study. Rarely observed fibroma, adenoma and gynecomastia and breast cancer in male were excepted from the present study.

### 2. The determination of urinary estrogens.

The chemical determination was carried out because of the above described reason. Though the colorimetric methods based upon the KOEHLER reaction for the chemical determination of urinary estrogens have been proposed by several investigators, the urine of normal males and females contains such small concentrations of estrogens that these methods were usually insufficient in sensitivity. These methods necessitate large quantities of urine, so that nonspecific urinary chromogens interfere with the accurate determination, giving unfavorable condition for the microanalysis of general examination.

Recently, a number of fluorometric methods for the estimation of estrogens have been reported with the excellent sensitivity (JAILER, FINKELSTEIN et al, BATES and

COHEN, ENGEL et al and others).

Based upon these reports, the optimal conditions for maximum fluorescence of the pure crystalline estrone were determined with a PFALTZ & BAUER fluorophotometer and the urinary estrone-estradiol fraction was estimated with a modification of JAILER's method. As the details were reported in Saishin-igaku, Vol. 9, No. 6, 1954, in Japanese, the abstract will be noted below.

1). Extraction procedure of estrone-estradiol fraction from urine.

40cc of a 24 hour urine specimen are hydrolyzed with 6cc of concentrated HCl, boiled for 30 minutes and cooled rapidly. The hydrolyzed urine is saturated with NaCl and transferred to a separatory funnel, and extracted once with 60cc of benzene. The benzene extract is washed once with 10cc of distilled water, three times 20cc of 9 per cent  $\text{Na}_2\text{CO}_3$ , once 10cc of diluted  $\text{H}_2\text{SO}_4$  (4:5) and once again 20cc of distilled water. The washed extract is extracted twice with 20cc of 2N-NaOH. The alkaline extract is acidified to  $\text{pH } 8.0 \pm 5$  with concentrated HCl and then extracted once with 30cc of ether. The ether extract is washed twice with 20cc of 9 per cent  $\text{Na}_2\text{CO}_3$  and once 10cc of water. A 20cc aliquot of ether extract is evaporated to dryness in a test tube. The fluorescence reaction is carried out for the present dry residue.

The recoveries of estrone from water and urine by this procedure were about 75 per cent.

2). Fluorescence reaction and fluorometric measurement.

1cc of 70 per cent (by volume)  $\text{H}_2\text{SO}_4$  is added to the dry specimen, and heated in a water bath at  $80^\circ\text{C}$ . for 20 minutes, cooled, and diluted with 14cc of 65 per cent (by volume)  $\text{H}_2\text{SO}_4$  and measured immediately.

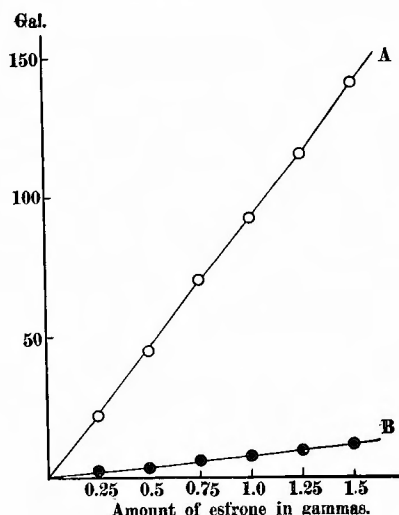
The fluorescence was read in a 15cc cuvette with a PFALTZ & BAUER fluorophotometer using the combination filters of Corning 5113 plus 3389 with maximum transmission at  $435 \text{ m}\mu$  as the lamp filter and the combination filters of Corning 3486 plus 9780 with maximum transmission at  $540 \text{ m}\mu$  as the photocell filter, the instrument being set to give a reading of 35 for the  $\text{B}_2$  permanent standard glass. For blank readings the measurement was repeated using a Corning 5840 filter with maximum transmission at  $365 \text{ m}\mu$  as the lamp filter, the fluorescent standard being set up at a reading of 8. The estrone of known amount added to water and extracted as described was applied as the standard, because of the incomplete recovery in described extraction procedure.

3). Correction for nonspecific fluorescence.

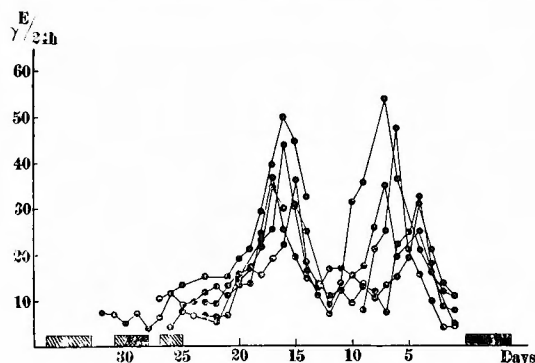
When the described fluorescence reaction is applied to the urinary extract, a green fluorescence due to estrogens as well as a somewhat pale-bluish fluorescence is observed due to some impurities which are impossible to remove by fractionation. For greater specificity an interference selective filter transmitting in the region of  $524 \text{ m}\mu$  as photocell filter is recommended to avoid this blue nonspecific fluorescence by BATES and COHEN. However, as the present interference filter has not been readily obtained in our laboratory, two filter combination systems were adopted according to the correction method of JAILER. Namely, the maximum fluorescence with crystalline estrone occurs with the exciting light of  $435 \text{ m}\mu$  as shown in Fig. 1,

**Fig. 1** Relationship between concentration of estrone in gammas and galvanometer readings with two filter combinations.

| Filter combinat. | Lamp filter                                                               | Photocell filter                                                          |
|------------------|---------------------------------------------------------------------------|---------------------------------------------------------------------------|
| A                | 5113 plus 3389 Corning filter (with maximum transmission at 435 m $\mu$ ) | 3486 plus 9780 Corning filter (with maximum transmission at 540 m $\mu$ ) |
| B                | 5840 Corning filter (with maximum transmission at 365 m $\mu$ )           | 3486 plus 9780 Corning filter                                             |



**Fig. 2** Daily urinary excretion of estrogen throughout the menstrual cycle in normal females.



etion showing 30~60  $\gamma$ , one at midinterval considered to bear some relationship to ovulation and the other preceding menses considered to be height of corpus luteum function. The present findings are in agreement with results reported by the various investigators, as shown by bio-assay. The values for the urinary excretion of estrogens obtained by bio-assay vary, due to differences in the technique employed

the galvanometer reading decreasing to practically zero at 365 m $\mu$ , while, the reagent blank extracted from distilled water as well as the extracts from infant urine which considered to contain very small amount of estrogens were distinctly determined to show the nearly equal reading at these two wave lengths. Thereupon, assuming the reading at 365 m $\mu$  to be due to the fluorescence of impurities except estrogens, the following formula was adopted for the correction factor:

$$\text{Estrogens } \gamma/24 \text{ h (Estrone-estradiol)} = \frac{\text{unknown } A - B}{\text{standard } \frac{1}{c} (A - B)} \times \frac{U}{40}$$

A: Galvanometer reading at 435 m $\mu$

B: Galvanometer reading at 365 m $\mu$

C: Amount of crystalline estrone used for standard ( $\gamma$ ).

U: Amount of through 24 hour urine specimen (cc).

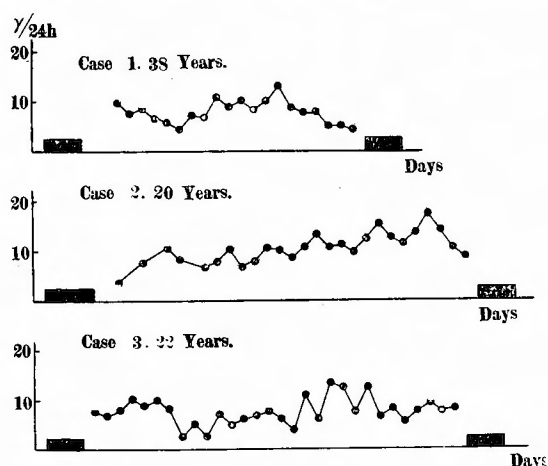
The suitable range of estrone concentration measured with above described method was between 0.2  $\gamma$  and 1.8  $\gamma$ , the mean experimental error being about  $\pm 10\%$ .

3. Urinary estrogen excretion in normal women and various normal conditions.

The daily urinary excretion of estrogens in six normal women (aged 20~42) with normal menstrual cycle was studied through the menstrual cycle by the present fluorometric method. The results are shown in Fig. 2. It can be seen that there are two peaks of excretion

by the various investigators. However, it is demonstrated that according to JAILER's report summing various references, generally, the equivalent of approximately 5~20  $\gamma$  of estrone as determined by bio-assay are excreted daily by the normal female between the peaks, at which time the excretion may rise to as high as 60~100 $\gamma$ . The present values of the author are considered somewhat lower than these values by bio-assay. This is considered because estriol was abandoned and estradiol, having remarkably strong biological activity than estrone, was measured taking estrone as the standard. The values obtained by JAILER with the fluorometric method are completely in accord with those obtained by the author.

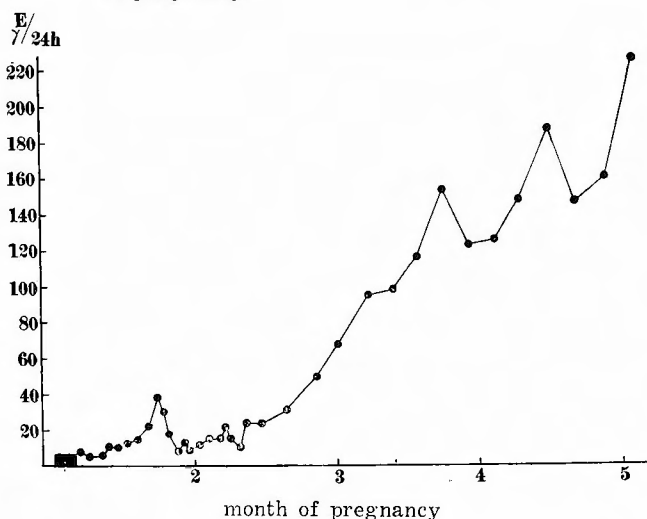
However, it was observed that the excretion of estrogens throughout the menstrual cycle in a married woman with normal menstrual cycle, aged 38, suffering from sterility was definitely atypical in that the peak of estrogen excretion was absent; and two unmarried young women with the regular menstrual cycle, suffering from dysmenorrhea showed also atypical curve without distinct peak (Fig. 3). These findings are considered to be due to an ovarian dysfunction of non-ovulatory type. Furthermore, it was observed that the daily urinary excretion in normal women in the menopause showed usually low value of about 5  $\gamma$ , although a few showed sometimes too low value impossible to measure; and one multipara, aged 59, 10 years past the menopause showed a flat curve of estrogen excretion with a continuous low value of about 5  $\gamma$  during the period of



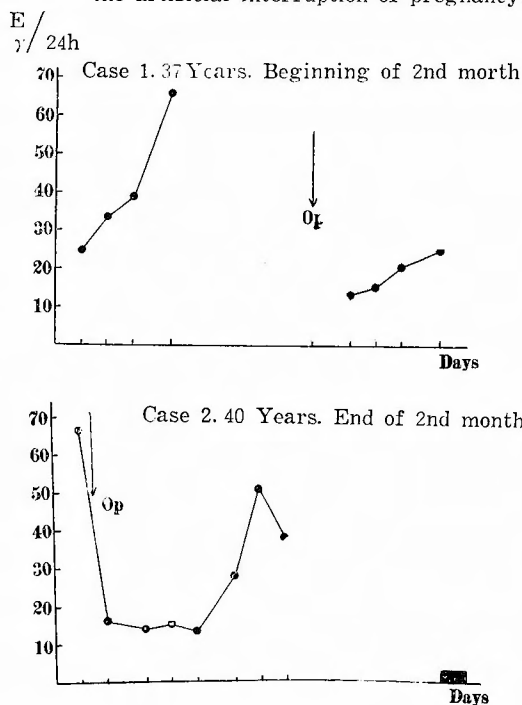
about one month. These findings are almost in accord with those obtained by JAILER. However, it is considered that such low value is the minimum limit obtained by the described method, because of the interference with impurities in urine specimen showing lower value than 5  $\gamma$ , though the above-described correction is applied.

On the other hand, in pregnancy urine containing high amount of estrogens, the values obtained were 116  $\gamma$  per day at the 3rd month, 192  $\gamma$  at the 4th month and 493  $\gamma$  at the 8th month; furthermore, the estrogen excretion in a pregnant woman was observed from the month in which being pregnant through the end of following four months. The result showed a distinct increasing at the period as early as the 2nd month of pregnancy, as shown in Fig. 4. Amounts of urinary estrogens measured before as well as after the operation of artificial interruption of pregnancy of two cases are showed in Fig. 5, recovering relatively normal value immediately after the operation. Furthermore, the values obtained in stallion

**Fig. 4** Urinary estrogen excretion during the first half of pregnancy.



**Fig. 5** Urinary estrogen excretion before and after the artificial interruption of pregnancy.



urine of two cases, considered to contain a remarkably large amount of estrogens, were 11 125  $\gamma$  and 11144  $\gamma$  per liter respectively.

It may be considered that the results of the foregoing observations were made available to show the possible clinical application of the above described method.

4. Discussion on determination of estrogens measured.

As already mentioned, the urinary excretion of estrogens in female shows a considerable variation, correlating with a rise and fall in ovarian activity during the menstrual cycle. Two peaks in the above shown monthly curve of estrogen excretion in normal women, seen at the time of ovulation and at the height of corpus luteum function, showed abrupt rise and sharp fall, and the peak value showed multifold figure against the minimum value in the daily excretion. On the other hand, the menstrual cycle of a normal woman may frequently have a variation of two or three days in the month. Accordingly, it is considered to be impossible, in general, to determine clearly through several measurements in a month at the temporary period or even considering the menstrual cycle, except pregnancy urine containing remarkably large amount.

Thereupon, in women having the menstruation daily measurement was continued throughout about one menstrual cycle, and the configuration of the monthly excretion curve was observed carefully since the irregularity of excretion curve was considered more significant to be aware of ovarian function. Nevertheless, in cases

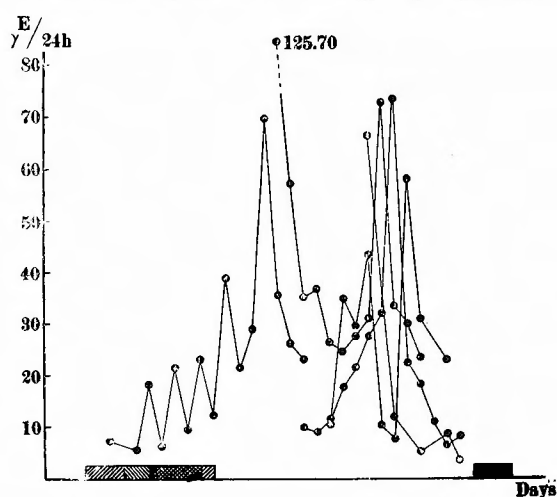
showing the peak on the excretion curve, the peak values of higher than  $60\gamma$  and lower than  $30\gamma$  were determined as the high and the low respectively, although it is remarkably difficult to determine the amount by the figures.

### III. URINARY ESTROGEN EXCRETION IN PATIENTS WITH NEOPLASTIC DISEASES OF THE BREAST

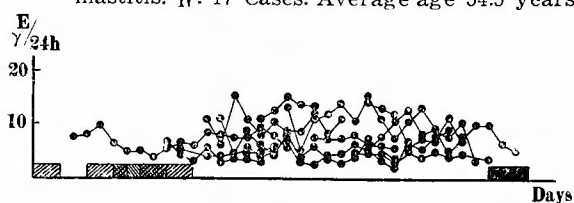
#### 1. Chronic cystic mastitis (mastopathia chronica).

Studies on urinary excretion of estrogens have been carried out in 50 patients with chronic cystic mastitis, in three of them menses being already absent. In the menstruating 47 cases, the configuration of the monthly curve of estrogen excretion was observed in the first place. And it was revealed that in 26 among 47 cases (55.3%) the monthly curves resembled those of normal women, while the remainder (44.7%) showed definitely atypical curves. In 26 cases showing typical curves with two peaks, the peak

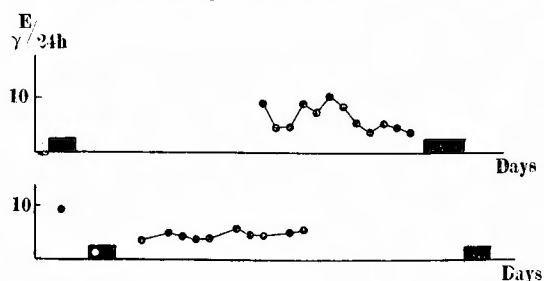
**Fig. 8** Urinary estrogen excretion in chronic cystic mastitis. III. 5 Cases. Average age 40.2 years



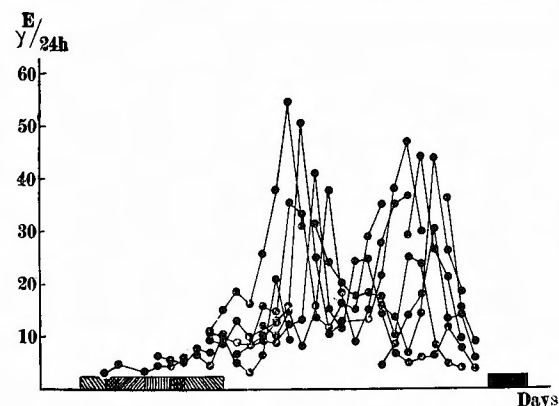
**Fig. 9** Urinary estrogen excretion in chronic cystic mastitis. IV. 17 Cases. Average age 34.5 years.



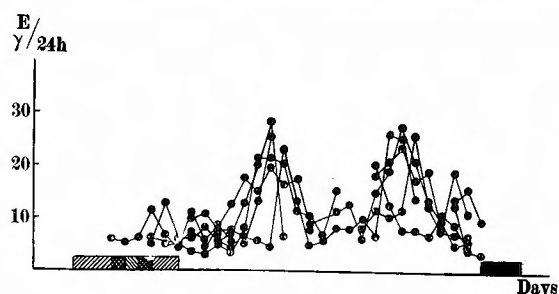
**Fig. 10** Urinary estrogen excretion during two menstrual cycles in a patient with chronic cystic mastitis.



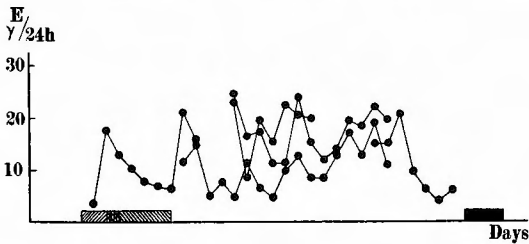
**Fig. 6** Urinary estrogen excretion in chronic cystic mastitis. I. 11 Cases. Average age 39.5 years.



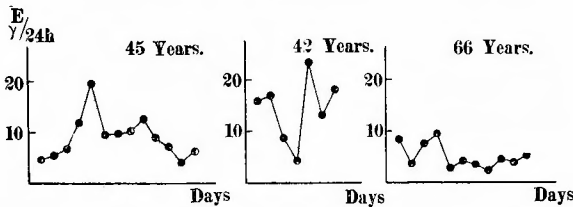
**Fig. 7** Urinary estrogen excretion in chronic cystic mastitis II. 10 Cases. Average age 39.4 years.



**Fig. 11** Urinary estrogen excretion in chronic cystic mastitis. V. 4 Cases. Average age 40.7 years.



**Fig. 12** Urinary estrogen excretion in 3 patients in the menopause with chronic cystic mastitis.



(Fig. 10). In remaining 4 cases, the monthly curves were irregular although the daily excretion levels were sometimes higher than 20  $\gamma$  (Fig. 11). In 3 cases with amenorrhea the daily excretions were found to vary between 5 and 20  $\gamma$  (Fig. 12).

In interpreting the described results from the configuration of the curve and daily excretion levels, mere 23.4% of total cases were normal and 58.3% of abnormal cases showed atypical curves without the peaks. The daily excretion levels were normal or slightly low in the majority of cases although mere 10.6% of cases showed high peak value.

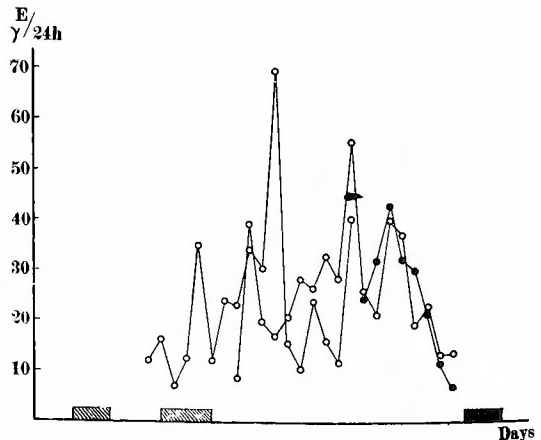
## 2. Carcinoma of the breast.

### 1). In the pre-menopause.

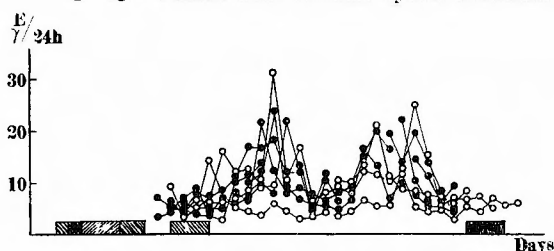
Studies on urinary excretion of estrogens have been carried out in 28 patients in the pre-menopause with breast cancer. Assays were continued throughout one menstrual cycle at the least. In 12 of a total of 28 cases (42.9%), the configuration of the monthly curve of excretion resembled those of normal controls. But the peak values were normal or slightly high in mere three cases (10.7%), two of which being complicated with chronic cystic mastitis (Fig. 13),

values were normal in 11 cases (23.4%) (Fig. 6), lower than 30  $\gamma$  in 10 cases (21.3%) (Fig. 7) and higher than 60  $\gamma$  in 5 cases (10.6%) (Fig. 8). In 21 cases showing atypical curves, the peaks ordinarily seen at the time of ovulation as well as at the height of corpus luteum function were absent in 17 cases (36.2%) and the daily excretions were somewhat slightly lower than those observed in normal subjects (Fig. 9). The study of one patient showing an atypical curve without two peaks was repeated after a 7 months' interval, the second determination showing a similar curve

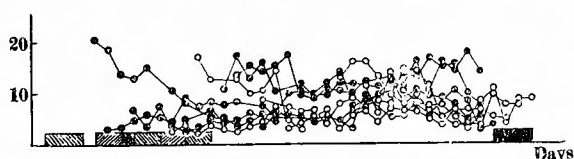
**Fig. 13** Urinary estrogen excretion in carcinoma of the breast. I. 3 Cases. Age 33, 47, and 55 years  
○—○ : Cancer with chronic cystic mastitis



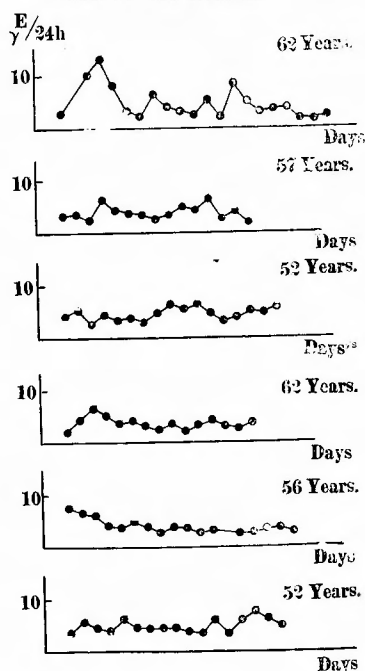
**Fig. 14** Urinary estrogen excretion in carcinoma of the breast. II. 9 Cases. Average age 44.9 years.  
○—○: Cancer with chronic cystic mastitis.



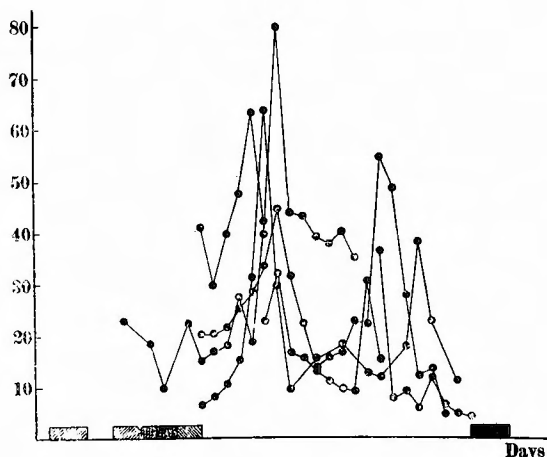
**Fig. 15** Urinary estrogen excretion in carcinoma of the breast. II. 15 Cases. Average age 42.1 years.  
○—○: Cancer with chronic cystic mastitis.



**Fig. 16** Urinary estrogen excretion in the menopausal patients with carcinoma of the breast.



**Fig. 17** Urinary estrogen excretion in fibroadenoma of the breast. 6 Cases. Average age 26.5 years.  
E/24h



while the remaining 9 cases (32.1 %) showed lower than 30  $\gamma$  (Fig. 14). Regarding the excretion cycle of 16 among 28 cases (57.1%) appeared atypical, the peaks of excretion usually observed in normal subjects were absent (Fig. 15).

Breast cancer with chronic cystic mastitis was present in 9 of 28 cases of breast cancer, estrogen excretion being illustrated in Figs. 13, 14 and 15. Namely, there was no significant difference between breast cancer with and without chronic cystic mastitis.

## 2). In the menopause.

Studies on 25 patients in the menopause with breast cancer showed essentially the same excretion rates as women in the same period without the disease (Fig. 16).

## 3. Fibroadenoma of the breast.

A study on 6 cases of fibroadenoma of the breast revealed that in the whole cases the monthly curve of excretion resembled those of normal women, while in 2 of 6 cases the peak values were slightly higher than the normal range (Fig. 17).

#### IV. SEX HORMONE BALANCE IN PATIENTS WITH NEOPLASTIC DISEASES OF THE BREAST

It is generally accepted at present that estrogens are capable of developing the duct systems in the breasts of man and animals. Lesions comparable to breast cancer or chronic cystic mastitis seen in the human being have been produced in the breasts of experimental animals by the long-sustained administration of estrogens. It may be expected, therefore, that the excessive estrogenic stimulation is the sole or direct etiological factor in the production of benign or malignant tumors of the human breast. However, as already mentioned, the urinary excretion levels of estrogens in 109 patients with neoplastic diseases of the breast were within normal limits in general, mere several cases showing relatively higher values. There remains the question, however, as to whether a state of hyperestrogenism occurs in a decrease in the secretion of androgens and progesterone, considered to be physiologically antagonistic to estrogens. Furthermore, it is suggested that these hormonal unbalance may be considered more significant in the genesis of neoplastic diseases of the breast.

For the purpose of clearing up these questions even to a slight extent, the ratio of urinary estrogens to 17-ketosteroids (17-KS) in 106 patients with neoplastic diseases of the breast was observed. Namely, simultaneous daily determinations of urinary estrogens and 17-KS (by Y. ISEDA in our laboratory) were continued throughout about one menstrual cycle to detect the accurate ratio of estrogens ( $\gamma$ ) to 17-KS (mg).

##### 1. In normal females.

The monthly curves of the urinary sex hormone balance (Estrogens/17-KS) in normal females resembled those of the normal estrogen excretion as shown in Fig. 18. Namely, it was found that there were two peaks in normal balance curves, one at midinterval and the other immediately before menses. The normal peak balance ranged from 3.5 to 6.0 and except for the peak balance, the averages of the hormone balance ranged from 0.5 to 2.0.

##### 2. In patients with neoplastic

Fig. 18 Urinary sex hormone balance throughout the menstrual cycle in normal females.

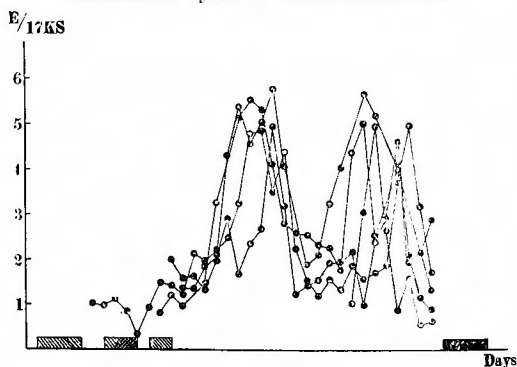
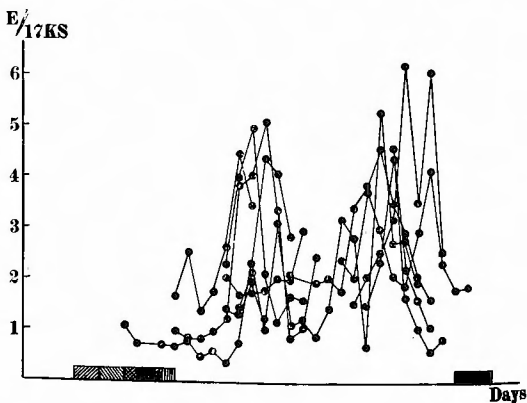
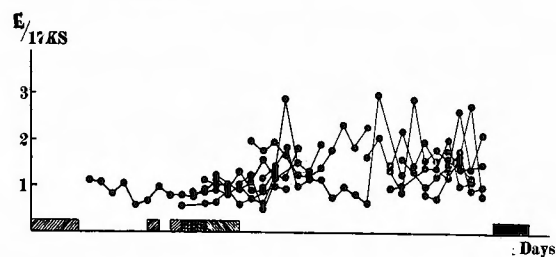


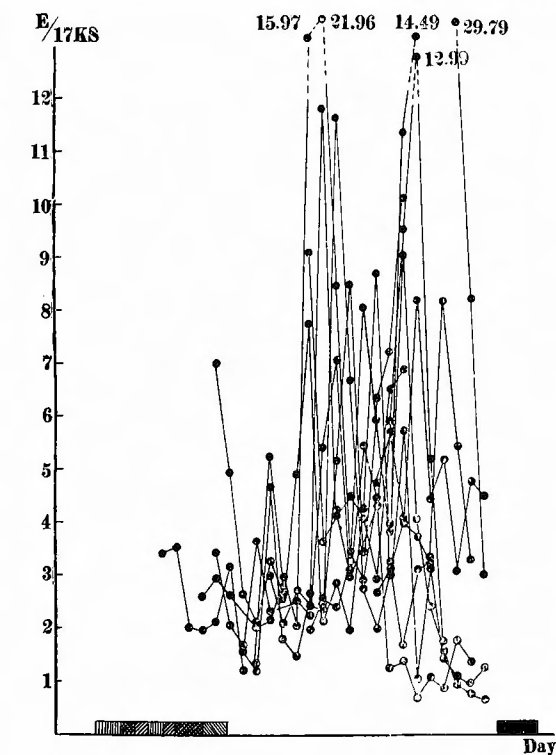
Fig. 19 Urinary sex hormone balance in chronic cystic mastitis. [ 10 cases; Average age 40 years.



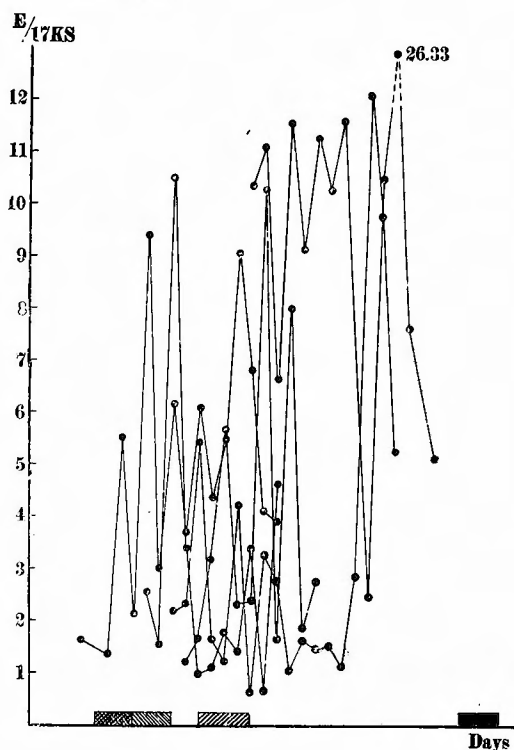
**Fig. 20** Urinary sex hormone balance in chronic cystic mastitis. II. 13 Cases. Average age 36.9 years.



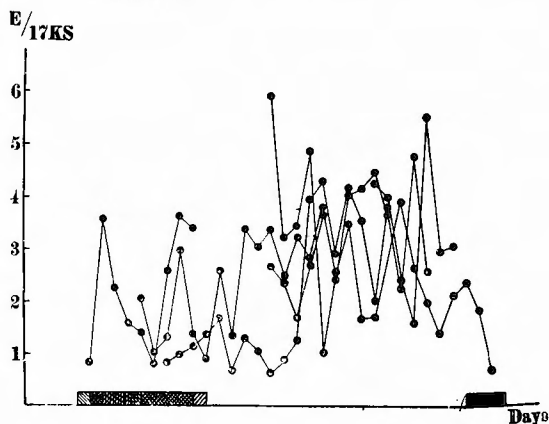
**Fig. 21** Urinary sex hormone balance in chronic cystic mastitis. III. 14 cases. Average age 39.8 years.



**Fig. 22** Urinary sex hormone balance in chronic cystic mastitis. IV. 5 Cases. Average age 44.8 years.



**Fig. 23** Urinary sex hormone balance in chronic cystic mastitis. V. 6 Cases. Average age 34.3 years.

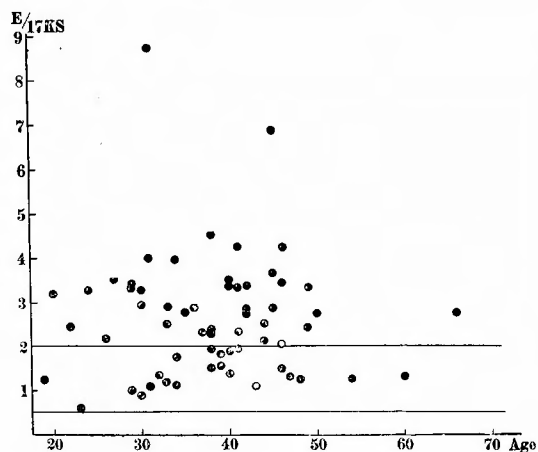


diseases of the breast.

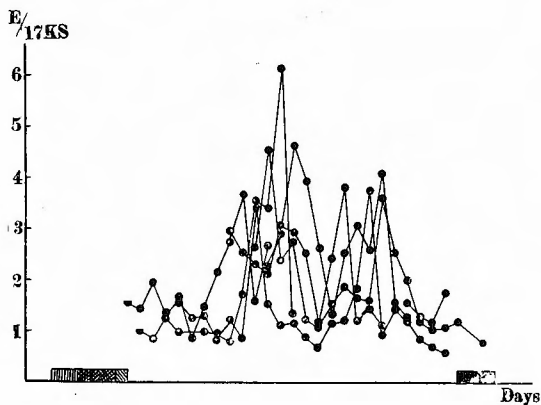
1). Chronic cystic mastitis.

Studies of urinary sex hormone balance have been carried out in 48 patients with chronic cystic mastitis. In mere 10 of a total of 48 cases (20.8%) the monthly curves of the hormone balance were definitely typical and the peak balance ranged within normal limits (Fig. 19). However, in the remaining 38 cases (79.2%) there were significant abnormality either in the configuration of the monthly

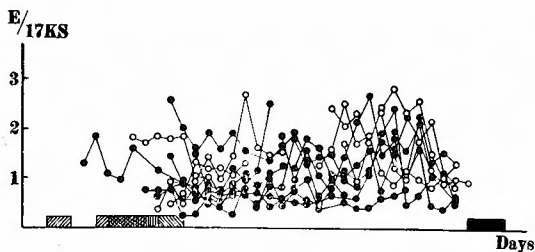
**Fig. 24** Averages of hormone balance in chronic cystic mastitis, excluding peak balances. (62 cases)



**Fig. 25** Urinary sex hormone balance in carcinoma of the breast. I. 6 Cases. Average age 47.0 years



**Fig. 27** Urinary sex hormone balance in carcinoma of the breast. III. 13 cases. Average age 42.5 years  
○—○ : Cancer with chronic cystic mastitis



balance curve or in the peak balance. Namely, the peak balances were absent in 13 cases (27.1%, Fig. 20), the peak balances of regular balance curves were higher than 6.5 in 14 cases (29.2%, Fig. 21), and the balance curves were irregular in 11 cases (22.9%) of which 5 had higher peak balance (Fig. 22) and 6 had normal or slightly lower peak balance (Fig. 23).

Except for the peak balance, the averages of hormone balance were higher than normal in the majority of cases, as shown in

**Fig. 26** Urinary sex hormone balance in carcinoma of the breast. II. (menopausal group)

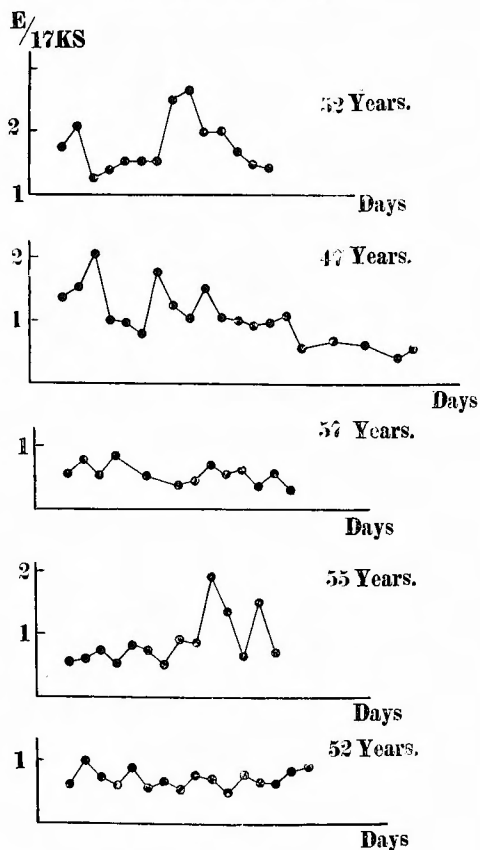


Fig. 24. Namely, the relative hyperestrogenism was present in 40 of a total of 62 cases (64.5%).

## 2). Carcinoma of the breast.

In carcinoma of the breast, the monthly curves of hormone balance were typical in 29 of a total of 52 cases (55.8%), that is, the regular balance curves with normal peak balances were present in 6 cases (11.6 %, Fig. 25) and the flat curves without definite peak were present in post-menopausal 13 cases (44.2%, Fig. 26). On the other hand, the remainder (23 cases, 44.2%) showed significant abnormality either in the configuration of the balance curve or in the peak balance. Namely, the peak balances were definitely absent in 13 cases (25.0%) which are still menstruating (Fig. 27), the peak balances of regular balance curves were higher than 6.5 in 5 cases (9.6%, Fig. 28), and the balance curves were irregular in 5 cases (9.6%) although there were sometimes the peak balances of normal range (Fig. 29).

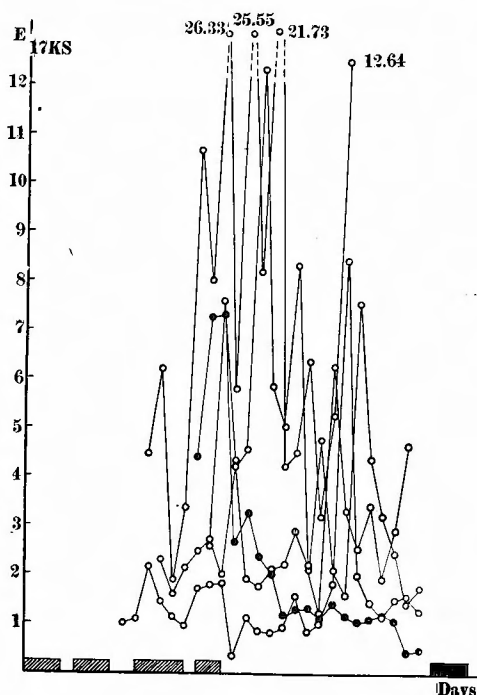
Breast cancer with chronic cystic mastitis was present in 9 of the 52 cases of breast cancer. In all of these cases, the sex hormone balances were abnormal as shown in Table 1, Figs. 27, 28, and 29 and resembled fairly closely those of chronic cystic mastitis.

Except for these cases, the sex hormone balances were generally normal in the majority of breast cancer (67.4%). It was observed that 4 in 5 cases, the peak balances showing higher than 6.5 in breast cancer, were complicated with chronic cystic mastitis.

Except for the peak balance, the averages of hormone balance were usually within the normal range (84.9%, Fig. 30), and 4 of 8 cases of relative hyperestrogenism were complicated with chronic cystic mastitis.

## 3). Fibroadenoma of the breast

**Fig. 28** Urinary sex hormone balance in carcinoma of the breast. IV. 5 Cases. Average age 38.8 years. ○—○ : Cancer with chronic cystic mastitis.



**Fig. 29** Urinary sex hormone balance in carcinoma of the breast. V. 5 Cases. Average age 44 years. ○—○ : Cancer with chronic cystic mastitis.

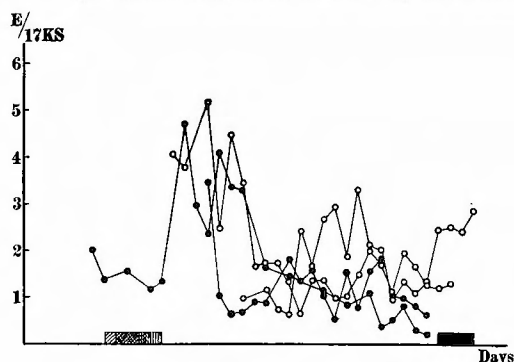


Table 1. Urinary sex hormone balances throughout the menstrual cycle in neoplastic diseases of the breast.

| Sex hormone balances                                    |                                                                   | Chronic cystic mastitis | Breast cancer | Fibroadenoma |
|---------------------------------------------------------|-------------------------------------------------------------------|-------------------------|---------------|--------------|
| Normal                                                  | Normal peak balance with regular balance curve                    | 10                      | 6             | 6            |
|                                                         | Absent peak balance with amenorrhea                               | 0                       | 23            | 0            |
| Abnormal                                                | Absent peak balance with regular menstruation                     | 13                      | 13(3)         | 0            |
|                                                         | Peak balance higher than 6.5 with regular balance curve           | 14                      | 5(4)          | 0            |
|                                                         | Peak balance higher than 6.5 with irregular balance curve         | 5                       | 0             | 0            |
|                                                         | Peak balance within the normal range with irregular balance curve | 6                       | 5(2)          | 0            |
| Totals                                                  |                                                                   | 48                      | 52(9)         | 6            |
| Averages of sex hormone balance, excluding peak balance | More than 2 (Relative hyperestrogenism)                           | 40                      | 8(4)          | 4            |
|                                                         | Normal                                                            | 22                      | 45(5)         | 11           |
| Totals                                                  |                                                                   | 62                      | 53(9)         | 15           |

( ) : Breast cancer with chronic cystic mastits.

Fig. 30 Averages of hormone balance in carcinoma of the breast, excluding peak balances. 53 cases.  
○ : Cancer with chronic cystic mastitis

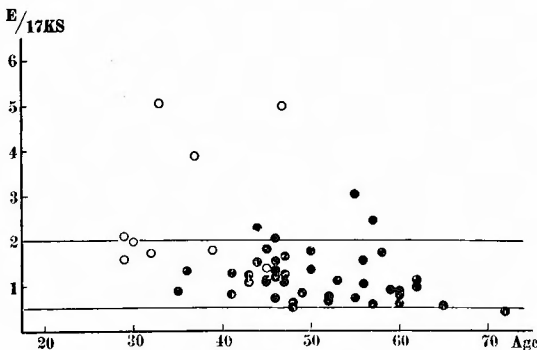
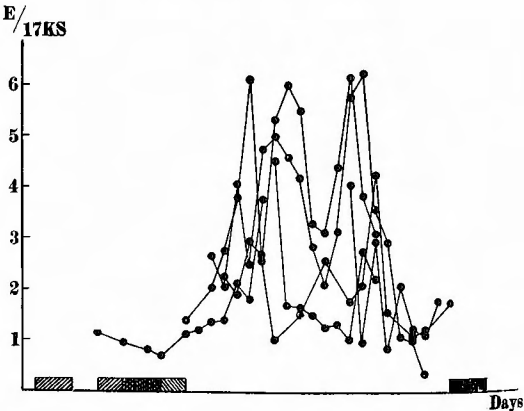
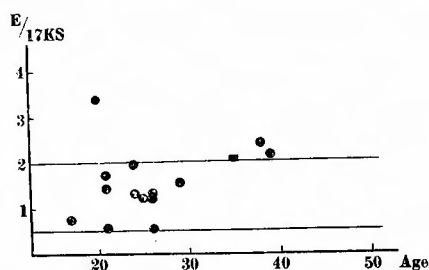


Fig. 31 Urinary sex hormone balance in fibroadenoma of the breast. 6 cases. Average age 26.5 years.



**Fig. 32** Averages of hormone balance in fibroadenoma of the breast, excluding peak balances. 15 cases.



In 6 cases of fibroadenoma of the breast, no gross abnormality was observed either in the configuration of the balance curve or in the averages of hormone balance (Figs, 31, 32). In 2 cases of urinary hyperestrogenism, sex hormone balances were as well within the normal range, because urinary hyperandrogenism was present at the same time.

## V. DISCUSSION

The relationship of the breast to the ovary has been recognized since the report of COOPER in 1829. Thereafter many have emphasized and extended this observation. It is generally accepted at present that estrogens play an important role in the development and the maintenance of function of the mammary gland. Therefore, in the endocrinological studies of neoplastic breast diseases estrogens have been noted in the first place by many investigators and have been widely applied in the experimental studies or in the treatment of these diseases. Nevertheless, relatively many studies on the conditions of estrogens in urine, blood and mammary tissue in the patients with these diseases have not yet appeared. Due to the comparative ease with which estrogens can be extracted from urine as compared to blood and tissue, the studies of urinary excretion have mostly been found. However, the results obtained were various in respective investigators, for instance, WANKE has reported the decreased excretion of estrogens in several patients with chronic cystic mastitis, while TAYLOR, BUCHER and GESCHICKTER could find no gross abnormality in the total output of estrogen excretion in these patients. NATHANSON studied the cycles of estrogen excretion in their patients and noted atypical curves in the certain type of chronic cystic mastitis. On the other hand, FUJIMORI recently made several determinations of estrogens in blood on each of their patients and reported the increased levels in the majority of them. These great discrepancies may possibly be due to differences in the technique of bio-assay employed by the various investigators. However, in addition to this, the differences in the point of observation are considered more significant. As stated previously, the excretion of estrogens are closely related with the menstrual cycle, and on account of the variation in excretion at different times in the monthly cycle, the method of observation applied by respective investigators was various. Regarding the present subject, it was believed that observations in the cycles of excretion are most valuable in the interpretation of ovarian function, consequently observations in the present paper were stressed on the configuration of the excretion curve.

It is likely to have fairly well established at present that in addition to estrogens, androgens, progesteron, hormones of the adrenals and hormones of the pituitary gland may be closely related as well to the development and function

of the mammary gland. It is generally considered that some of these hormones are physiologically antagonistic to estrogens. Therefore, by the reason as stated previously, studies of urinary sex hormone balance (Estrogens/17-KS) in patients with neoplastic diseases of the breast were carried out as well in our laboratory. Such continuous daily determinations of urinary sex hormone balance in these patients have not yet been reported.

It is first noted in the data obtained that the presence of urinary hyperestrogenism was unexpectedly small either in the benign or malignant breast disease. This finding indicates that a state of hyperestrogenism in patients with neoplastic breast diseases should not be expected directly through the results of animal experiments by prolonged administration of estrogens of over a physiologic amount.

In fibroadenoma of the breast, the monthly curve of estrogen excretion was definitely normal although a few showed slightly higher peak values, and the sex hormone balance was also usually within the normal range. Namely, there is no indication of a dysfunction of the ovary and a faulty steroid metabolism. Fibroadenomas are found most frequently in young women of the second and third decade. Therefore, they may represent a more localized response to a physiologic amount of the hormone.

In chronic cystic mastitis, the monthly curve of estrogen excretion was definitely atypical in 44.7% of cases. It was found that the peaks ordinarily observed at the time of ovulation and at the height of corpus luteum function were most frequently absent. It is probably due to an ovarian dysfunction, in which the normal cyclic secretion of estrogens is absent. It is difficult to determine whether such abnormalities were constant from month to month. However, since it is evident that a cyclic change occurs in the breast, which can be correlated with a rise and fall in ovarian activity during the menstrual cycle, it is possible that such abnormalities disturb a cyclic histological change in the breast. Furthermore, it is also possible that if such abnormalities are frequently repeated, pathological changes will finally occur in the breast tissue. Recently, KIER and associates reported abnormal endometrial morphology suggesting ovarian dysfunction of a non-ovulatory type in a large number of patients with benign breast lesions. This report is likely to be in accord with ours on a different standpoint.

In 55.3% of cases of chronic cystic mastitis, however, the configuration of the excretion curve resembled those of normal controls and daily excretions, especially the peak values, were usually normal or slightly lower although a few showed higher values. It is difficult to find a state of ovarian function through these data. However, in studies of urinary sex hormone balance in these patients, the monthly curves were definitely atypical in a high percentage of cases (80%) and the averages of hormone balance showed a state of relative hyperestrogenism due to urinary hypoandrogenism in the majority of cases. When such an unbalance of sex hormone continues, even though the secretions or excretions of estrogens are normal, it may react upon susceptible breast tissue as the abnormal stimuli. Although it is dangerous to draw the conclusion directly through the foregoing data, it can be stated at least

that ovarian dysfunction of a non-ovulatory type and the presence of a hormonal unbalance may be a factor in the production of chronic cystic mastitis.

According to the reports appeared, it has been stated that the estrogen excretion in patients with breast cancer was generally normal. In contrast to these findings, atypical curves of estrogen excretion, the peak of excretion being absent, were found in the majority of pre-menopausal patients with breast cancer in our clinic. Furthermore, in cases showing typical curves the daily excretion, especially the peak value, was usually slightly low. Such a state which may be probably due to an ovarian dysfunction was found in more higher percentage than in chronic cystic mastitis. In regard of the estrogen excretion alone, no significant difference was observed between chronic cystic mastitis and breast cancer in pre-menopausal women. Hereby, it must be pointed out that the age incidence in these patients resembled those of chronic cystic mastitis, that is, the average age of these patients was 42.1 years and those with chronic cystic mastitis 38.6. It is possible, however, that in carcinoma of the breast a disturbance of ovarian function or a faulty metabolism of estrogens may occur secondarily as an effect of the appearance of malignant lesion. Therefore, it may be dangerous to draw the conclusion directly for the relationship between chronic cystic mastitis and breast cancer through these data.

On the other hand, although urinary sex hormone balance in breast cancer with chronic cystic mastitis resembled those in chronic cystic mastitis, the hormone balances in breast cancer without chronic cystic mastitis were usually normal in contrast to those in chronic cystic mastitis. These discrepancies were due to the differences of urinary excretion of 17-KS between these two conditions.

It is clear that urinary excretion levels of the sex hormone represent only the end products of metabolism. It has not yet been proved whether or not they give a true index of the blood levels, the rate of secretion or destruction, or the utilization of the hormones by the tissues. Furthermore, they do not reveal the exact nature or relationship of the various components of the hormones excreted.

From the foregoing data, however, it can be concluded that in all types of neoplastic diseases of the breast observed, there is a suggestion of some dysfunction of the endocrine system as a cause or effect in the production of these lesions. This may be particularly emphasized in chronic cystic mastitis. It must be stated at present that the problem of possibility of malignant transformation of chronic cystic mastitis is endocrinologically still equivocal. Nevertheless, it is believed that chronic cystic mastitis should be carefully treated as precancerous changes.

## VI. SUMMARY

A fluorometric method for the clinical determination of estrogens (estrone plus estradiol) in urine was presented and the urinary excretion of estrogens in 109 patients with neoplastic diseases of the breast was determined by this method. At the same time, the ratio of urinary estrogens to 17-KS in these patients was

observed as well. The results obtained were as follows :

1). The presence of urinary hyperestrogenism was unexpectedly small in both the benign and malignant breast diseases.

2). In fibroadenoma of the breast, there was no definite abnormality either in the estrogen excretion or in the sex hormone balance (Estrogens/17-KS).

3). In chronic cystic mastitis, the monthly curves of estrogen excretion and daily excretion were normal in mere 23.4% of cases and atypical curves of estrogen excretion that are probably due to an ovarian dysfunction of a non-ovulatory type were found in the majority of abnormal cases. The monthly curves of sex hormone balance in these patients were definitely atypical in a high percentage of cases and the averages of hormone balance, excluding peak balances, showed usually a state of relative hyperestrogenism due to urinary hypoandrogenism.

4). In carcinoma of the breast in post-menopausal patients, no significant variation was observed in the estrogen excretion. However, in per-menopausal patients, atypical curves of estrogen excretion, the peak of excretion being absent, were found in the majority of cases. On the other hand, the monthly curves and the averages of sex hormone balance in these patients were usually normal.

5). In breast cancer with chronic cystic mastitis, the estrogen excretion and the sex hormone balance were similar to those of chronic cystic mastitis.

6). From the results of these observations, it can be concluded that an ovarian dysfunction of a non-ovulatory type and the presence of a hormonal unbalance may be an etiological factor in the production of certain types of chronic cystic mastitis. However, it must be stated that the relationship between chronic cystic mastitis and breast cancer is endocrinologically still equivocal.

Finally, the author wishes to thank Dr. K. MASUDA, lecturer of our clinic for his helpful guidance during the course of the present work, and to express his gratitude to Prof. Dr. U. MIURA and Assist-Prof. Dr. M. FUJIWARA of the Department of Hygiene, of our University for the loan of valuable fluorophotometer as well as highly appreciated advices on the fluorometry of urinary estrogen.

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## 和 文 抄 録

# 尿中 Estrogen の排泄状態より観た乳腺腫瘍特に マストパチー Mastopathie の内分泌学的研究

京都大学医学部外科学教室第2講座（指導 青柳安誠教授）

大学院学生 西 谷 奎 吾

Fluorometry による尿中 Estrogen (Estrone-Estradiol 分割) の化学的定量法を自ら改良し、その臨床的応用価値のあることを確認して、109例の乳腺腫瘍患者特に前癌状態として注目され且最近その発生数が増加して来たマストパチーを中心として、その尿中 Estrogen の排泄状態を観察し、更に尿中 17-Ketosteroids とのバランスの状態をも併せ観察して次のような結果を得た。

1. 乳腺腫瘍患者全体を通じ、良性、悪性を問わず Estrogen 排泄過剰を認めたものはごく少数に過ぎなかつた。

2. 乳腺線維腺腫に於ては、月経周期間の Estrogen の排泄曲線及び日々の排泄値共に略々正常で、少数例に於てやゝ高値を認めた。その両性ホルモンのバランスも亦略々正常であつた。

3. マストパチーでは、Estrogen の排泄曲線及び日々の排泄値共に全く正常のものは23.4%に過ぎず、異常なもの約60%は排泄曲線が非典型的で無排卵性の卵巣機能不全を思ひしめた。その両性ホルモンのバ

ランス (Estrogens/17-Ketosteroids) は高いもの即ち相対的 Estrogen 過剰と見做されるものが過半数を占め、バランス曲線の異常なものが約80%に認められた。

4. 乳癌の Estrogen 排泄状態は閉経後のものでは正常人と大差がないが、閉経前のもものでは過半数は排泄曲線の異常を認め、排泄曲線が正常に近いものでも稍低値を示すものが多かつた。その両性ホルモンのバランスは正常範囲のものが圧倒的に多く、そのバランス曲線もマストパチーから移行したと思われる乳癌を除いては略々正常に近いものが多かつた。

5. 即ちマストパチーから移行したと考えられる乳癌では Estrogen の排泄状態及び両性ホルモンのバランスの状態は共にマストパチーの夫とよく似ていて差異が認められなかつた。

6. 以上の結果から、無排卵性の卵巣機能不全並びに相対的 Estrogen 過剰というステロイドホルモンの代謝異常が或る種のマストパチー発生の一因子として考えられる。