New Methods of Laboratory Testing for the Sensitivity of the Uterus to Ovarian Hormones

By

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INTRODUCTION

A few investigators\(^{1-7}\) have reported that abnormal sensitivity of the uterus to ovarian hormones exists among the endocrine disorders which are regarded as due to endocrinological disturbances. However, generally test measuring of hormones has been undertaken and the dosages of hormone preparations have been applied in diagnosing and treating endocrine disorders, and the problem on the sensitivity of the uterus has been nearly neglected. Abnormal sensitivity of the uterus is assumed to occur, either dependently or independently of endocrine disturbances of the sex hormones.

In the present studies, the author attempted to elucidate the problem of the sensitivity of the human uterus to ovarian hormones by the author's new idea and succeeded in finding new methods of laboratory testing for a clinical diagnosis of the sensitivity of the uterus to ovarian hormones (uterus-sensitivity hereafter).

Clinical cases of uterine amenorrhea caused by hyposensitivity of the uterus

The author will report three cases of uterine amenorrhea which is caused by hyposensitivity of the uterus to sex hormones to prove the existence of a hyposensitive uterus.

The first case: A twenty-six-year-old, unmarried, Japanese, non-gravida, complaining of primary amenorrhea.

On pelvic examination, external and internal genitalia were normal. Tentative curettage of endometrium was undertaken, which was unsuccessful to gain. A culture of tuberculous bacilli of a vaginal smear was negative. The basal body temperature (B.B.T. hereafter) revealed a typical biphasic curve (Fig. 1.a). The measured value of various kinds of steroid in the urine proved that the ovarian cycle was normal (Fig. 2.a). The thyroid and liver function
Fig. 1, a. B.B.T. of the first case.

Fig. 1, b. B.B.T. of the second case.

Fig. 1, c. B.B.T. of the third case.
Fig. 2. B.B.T. and steroids in urine in three cases of uterine amenorrhea. (a) the first case (b) the second case (c) the third case

were normal. In order to confirm the normal ovarian cycle, a tentative laparotomy was done on the high-temperature phase of her B.B.T. curve. Corpus luteum was found in the right ovary and endometrium was also found in the small rudimentary uterine cavity which showed no sign of menstrual bleeding. A histological picture of the endometrium showed no progestational one, but atrophic, in spite of the B.B.T. demonstrating the luteal phase (Fig. 3).

Fig. 3. Histological picture of the endometrium of the first case.
The second case: A twenty-four-year-old Japanese, para 1, gravida 1, complaining of secondary amenorrhea occurring after her delivery. On pelvic examination, the external genitalia and uterus were normal. The B.B.T. showed a typical biphasic curve (Fig. 1,b).

The measured values of various kinds of steroid in the urine proved a normal functioning of the ovary (Fig. 2, b). Endometrium was quite difficult to gain by curettage.

The third case: A twenty-three-year-old, Japanese, non-gravida, complaining of primary amenorrhea. On pelvic examination, the external genitalia and uterus were normal. The histological picture of endometrium demonstrated a proliferative phase, in spite of a high-temperature phase on the B.B.T. curve which was typically biphasic (Fig. 1, c), and the measured value of steroid in the urine proved that the ovarian cycle was normal (Fig. 2,c). $^{32}$P-uptake test through endometrium (mentioned below) demonstrated the hyposensitivity of this uterus.

In all these three cases of uterine amenorrhea, the ovarian function was normal, but the uteri showed no reaction to ovarian hormone, that is, these uteri are supposed to be hyposensitive to ovarian hormone and deserve being referred to as hyposensitive uteri. There may be other hyposensitive uteri than these three cases of marked hyposensitivity, and new methods of laboratory testing are needed for the anomalous sensitivity of the uterus.

**New methods of laboratory test for the uterus-sensitivity**

The author devised new methods for laboratory testing for the uterus-sensitivity as follows:

1) Uterus-sensitivity test by $^{32}$P-uptake through endometrium

   A) Fundamental experiment (uterus function test)

   1) Method

   0.1 ml of sodium phosphate solution (pH 7.4) labelled with $^{32}$P which was contained in the ratio of 2 μc per kg of body weight was infused into the uterine cavities of the subjects through the vagina with a syringe, then, 1 ml of blood was sampled from their cubital vein, 15, 30 and 60 minutes thereafter to measure the radio-activity with a scillation counter (with head for β-ray) for 15 minutes. Their radio-activity was represented by counts per minutes (CPM hereafter) of arithmetic mean value.

   2) Results (Fig. 4)

   The subjects were divided into six groups. In normal subjects, the maximum value was attained in fifteen minutes ("rapid type"), the peak being higher in the cases in progestational phase (B) than those in the proliferative phase (A). The maximal values of senile uteri after menopause (C) and primary amenorrheic
uteri (D) were obviously lower than the normal, besides, retrogression of the peak
was observed in the former ("slow type"). In secondary amenorrheic uteri (E),
both types of the curve of $^{32}$P-uptake were observed. Hypoplastic uteri (F)
demonstrated the slow type of curve of $^{32}$P-uptake, but their values were gene-
really higher than in the cases of the senile uteri (C) and the primary amenorrheic
uteri (D).

**Fig. 4. Uterus function test by $^{32}$P-uptake through endometrium.**

- CPM per 1 ml of blood of the cases with various kinds of
  uterus, 15, 30 and 60 minutes after the infusion of $^{32}$P into
  their uterine cavity.

  Mean value of the above mentioned
  A: Normal uterus (proliferative phase)  B: Normal uterus (pro-
  gestational phase)  C: Senile uterus  D: Primary amenorrheic
  uterus  E: Secondary amenorrheic uterus  F: Hypoplastic
  uterus

**Evaluation of the results:**

From the results of $^{32}$P-uptake curve, a normal uterus was defined as fol-
 lows: It demonstrates the rapid type of the curve, the maximal value of the curve
being more than 20 CPM, and others were diagnosed as impaired uteri in their
Fig. 5. Evaluation of uterus function by $^{32}$P-uptake test in various kinds of uterus.

![Table showing evaluation of uterus function by $^{32}$P-uptake test.]

function. Illustrative data evaluated from this criterion are presented in Fig. 5.

B) Uterus-sensitivity test

1) Principle: The value of the $^{32}$P-uptake test may be affected by the change of hormonal circumstances. Therefore, the impaired function of the uterus, being detected by the $^{32}$P-uptake test, can be produced either by an impaired uterus itself or insufficient secretion of sex hormones. In order to find an impaired uterus in its sensitivity, the following method was undertaken.

2) Method: Uterus function test by the $^{32}$P-uptake curve was performed twice, directly before and twenty four hours after an intramuscular injection of 10,000 units of estradiol benzoate once a day for three successive days, and both values of the curve were compared.

3) Results (Fig. 6)

The subjects were divided into four groups. Most of normal uteri (A) represented marked elevation of the maximal values of the $^{32}$P-uptake curves after the administration of estrogen comparing with before the administration. In the cases of secondary amenorrheic uteri (C) and hypoplastic uteri (D), some of them represented the same changes of value of the $^{32}$P-uptake curve, but most of primary amenorrheic uteri showed no change in their $^{32}$P-uptake curve (B).

Evaluation of the results:

The uterus which showed the CPM value of more than 20 and an increase of more than 20% of CPM value at 15 minutes after infusion of $^{32}$P, after the administration of estrogen, in comparison to that before the administration of estrogen was diagnosed as to be normally sensitive, and others were diagnosed as a hyposensitive uterus. Illustrative data in this connection are shown in Fig. 12.

II) Uterus-sensitivity test by measurement of oxygen consumption of uterine tissue

A) Method
Fig. 6. Uterus-sensitivity test by $^{32}$P-uptake through endometrium.

- CPM per 1 ml of blood after infusion of $^{32}$P into the uterine cavity, before the administration of estradiol benzoate 1 mg daily for three days.
- Mean value of the above mentioned.
- CPM per 1 ml of blood after infusion of $^{32}$P into the uterine cavity, after the treatment above mentioned.
- - Mean value of the above mentioned.
A: Normal uterus (proliferative phase)  B: Primary amenorrheic uterus  C: Secondary amenorrheic uterus  D: Hypoplastic uterus

This test was begun from the third day after the completion of menstruation in the case of menstruating subjects, and from the arbitrary period in the case of amenorrheic subjects.

An endometrial specimen was sampled for the measurement of its oxygen consumption by curetage three times, i.e., the first, before the injection and the second, two days after the injection of 100,000 units of estradiol benzoate, which was followed by an injection of a mixed preparation of 250 mg of 17α-hydroxyprogesterone capronate and 10 mg of estradiol valerianate, the third, seven days after the second injection of the mixed preparation.

A myometrial specimen was taken at the same time of the second sampling of endometrium, by puncture-needle of Tsurumaru’s type, modified by the author, and its oxygen consumption was measured.
The procedure of measurement of the oxygen consumption: A Cartesian diver manometer was used in the present study as a chief apparatus for measurement of oxygen consumption. The operation of this manometer was carried out essentially by Holter’s method modified by S. Sugawara et al., which represented good agreement with the result obtained by Warburg’s method.

B) Results

Q_02-value of endometrium (Fig. 7): The Q_02-values of normal uteri were found to be higher than those of amenorrheic uteri, especially, primary amenorrheic uteri, and increased by the administration of estrogen and decreased by progesterone, while the Q_02-value of amenorrheic uteri was found to be low and showed little change by the administration of sex hormones.

Q_02-value of myometrium (Fig. 8): The Q_02-value of uterine muscle was perceptibly low in the senile uterus after menopause and hypoplastic uterus, and notably high in a fibroid uterus in comparison to a normal uterus.

Evaluation of the results: The uterus in which the Q_02-value of endometrium after the injection of estrogen was more than 2.6×10^{-2} μl/mg, was evaluated to be normally sensitive, and the others were evaluated to be hyposensitive.

Fig. 7. Uterus-sensitivity test by Q_02-measurement of endometrium.
B: two days after administration of estradiol benzoate 10 mg.
C: seven days after administration of estradiol valerianate 10 mg and 17α-hydroxyprogesterone capronate 250 mg.
The uterus showing $Q_{O_2}$-value more than $7.0 \times 10^{-2} \mu l/mg$ of uterine muscle tissue after the injection of estrogen was evaluated to be hypersensitive, and it was evaluated to be normosensitive and hyposensitive respectively, when the value was between $7.0 \times 10^{-2} \mu l/mg$ and $2.0 \times 10^{-2} \mu l/mg$ and below $2.0 \times 10^{-2} \mu l/mg$.

The illustrative data evaluated by these criteria mentioned above were shown in Fig. 12.

III) Uterus-sensitivity test by histological examination of the endometrium

A) Method

This test was begun immediately after the completion of menstruation in the subject with a normal menstrual cycle, and was begun in an arbitrary period in the subject of an amenorrheic uterus.

Endometrial specimens were sampled from subjects with normal menstrual cycles and of primary and secondary amenorrheic uteri, twice from each subject, i.e., before and after the injection of mixed preparation of 250 mg of 17$\alpha$-hydroxyprogesterone capronate and 10 mg of estradiol valerianate.

These endometrial specimens were sampled by curettage from the medial portion of the uterine fundus of each subject.

B) Results (Fig. 9)
Most of the uteri with normal menstrual cycles were changed into the progestational phase from the proliferative phase in their histological picture after the injection of the mixed preparation of estrogen and progesterone, while most of the amenorrheic uteri showed to be remaining in the proliferative phase even after the injection of estrogen and progesterone.

![Fig. 9. Evaluation of uterus-sensitivity by histological examination for endometrium in various kinds of uterus.](image)

- \( \Box \) cases changed into progestational phase seven days administration of estradiol valerianate 10 mg and 17\(\alpha\)-hydroxyprogesterone capronate 250 mg (normosensitive uterus)
- \( \square \) cases not changed into progestational phase seven days after administration of the above mentioned hormones (hyposensitive uterus)

Evaluation of the results: The endometrium of the uterus, in which any progestational finding in its histological picture was found after the injection of the mixed preparation of estrogen and progesterone, was evaluated to be normosensitive, otherwise to be hyposensitive.

The evaluated data from this criterion were shown in Fig. 12.

IV) Uterus-sensitivity test by crystallization of cervical mucus

A) Method

This test was begun from the third day after the completion of menstruation in subjects with a normal menstrual cycle, and from the arbitrary period in amenorrheic subjects.

Cervical mucus was taken with forceps, being careful not to stain with blood, and spread upon a slide and dried for microscopic examination.

This test was performed twice, i.e., directly before and 48 hrs. after intramuscular injection of 100,000 units of estradiol benzoate and their crystallizations were examined comparing both specimens. Their crystallizations were represented by an estimate of extent of the crystals varying from 0 to 5 in number, as shown in Table I.

Evaluation of the results: The uterus, of which cervical crystals showed difference of more than 2 in number of evaluation between two specimens was diagnosed as normosensitive and was diagnosed as hyposensitive, when the
TABLE I. Gradation of Crystallization of Cervical Mucus

<table>
<thead>
<tr>
<th>Grade</th>
<th>Crystallization of cervical mucus</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>no crystal observed</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>non-typical crystal partly observed</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>non-typical crystal entirely observed</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>typical crystal partly observed</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>typical crystal entirely observed</td>
<td>4</td>
</tr>
<tr>
<td>6</td>
<td>nucleus of crystal appeared</td>
<td>5</td>
</tr>
</tbody>
</table>

difference was below 2. Illustrative data, classified in this connection are represented in Figs. 10 and 12.

Fig. 10. Uterus-sensitivity test by crystallization of cervical mucus.

- ■ cases changed into high degree of crystallization of cervical mucus two days after administration of 100,000 units of estradiol benzoate (normosensitive)
- □ cases not changed into high degree of crystallization of cervical mucus two days after administration of 100,000 units of estradiol benzoate (hyposensitive)

V) Uterus-sensitivity test by withdrawal-bleeding

The tests described above were originally devised by the author, while many reports concerning this withdrawal-bleeding test, have been published. The author modified this test to apply for the laboratory test of uterus-sensitivity as follows.

A) Method: The subjects were injected intramuscularly with the mixed preparation of 10 mg of estradiol valerianate and 250 mg of 17α-hydroxyprogesterone capronate, directly after the completion of menstruation of the subjects with a normal menstrual cycle or in the arbitrary period in amenorrheic subjects, and then, their withdrawal-bleeding was observed and was represented by the Kupperman’s index modified by the author to apply for the test of uterus-sensitivity.

The withdrawal-bleeding index was obtained as follows: The time required for onset of bleeding in days, calculated from the day of injection of the mixed preparation of estrogen and progesterone to the first day of bleeding, was divided into the duration of withdrawal menstrual bleeding in days. The quotient ob-
tained was then multiplied by an estimate of the extent of bleeding, varying from 0 to 5, and 100. Thus, 1 bleeding referred to staining; 2 described a mild to moderate flow; 3 was considered as a diminished flow, but more than moderate; 4 designated a normal menstrual flow; and some cases were referred to withdrawal bleeding as being 5 (characterized by flooding or hypermenorrhea). The numerical figure of a withdrawal-bleeding index was determined by the following formula:

\[ W.I. = \frac{D}{T} \times E \times 100 \]

W.I.: Withdrawal-bleeding index
\( T \): Time of onset
\( D \): Duration
\( E \): Extent

B) Results

Evaluation of results: The uterus representing the withdrawal-bleeding more than 5 was diagnosed as normosensitive, and below 5 was diagnosed as hyposensitive. A summation of the result evaluated from this criterion was represented in Figs. 11 and 12.

All of the normal uteri were diagnosed as normosensitive from this test, and more than half of amenorrheic uteri were also shown to be normosensitive.

![Graph](image)

**Fig. 11.** Uterus-sensitivity test by withdrawal-bleeding after administration of estradiol valerianate 10 mg and 17\( \alpha \)-hydroxyprogesterone capronate 250 mg.

DISCUSSION

Seeing the current study on the problem of the impaired function of the uterus, the uterus-sensitivity to sex hormone has been nearly neglected, and only
Fig. 12. Comparison of the evaluation of uterus-sensitivity obtained by various kinds of laboratory testing method.

<table>
<thead>
<tr>
<th>Testing method</th>
<th>Percentage of grade of uterus-sensitivity (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>70</td>
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<tr>
<td></td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>100</td>
</tr>
<tr>
<td>32P-uptake test</td>
<td>Normal uterus: 24%</td>
</tr>
<tr>
<td></td>
<td>Secondary amenorrheic uterus: 31%</td>
</tr>
<tr>
<td></td>
<td>Primary amenorrheic uterus: 11%</td>
</tr>
<tr>
<td></td>
<td>Hypoplastic uterus: 1%</td>
</tr>
<tr>
<td>Ga measurement test for endometrium</td>
<td>Normal uterus: 12%</td>
</tr>
<tr>
<td></td>
<td>Secondary amenorrheic uterus: 5%</td>
</tr>
<tr>
<td></td>
<td>Primary amenorrheic uterus: 2%</td>
</tr>
<tr>
<td>Ga measurement test for myometrium</td>
<td>Normal uterus: 15%</td>
</tr>
<tr>
<td></td>
<td>Fibroid uterus: 2%</td>
</tr>
<tr>
<td></td>
<td>Senile uterus: 3%</td>
</tr>
<tr>
<td></td>
<td>Hypoplastic uterus: 1%</td>
</tr>
<tr>
<td>Histological test for endometrium</td>
<td>Normal uterus: 10%</td>
</tr>
<tr>
<td></td>
<td>Secondary amenorrheic uterus: 4%</td>
</tr>
<tr>
<td></td>
<td>Primary amenorrheic uterus: 5%</td>
</tr>
<tr>
<td>Cervical-mucus-crystallation test</td>
<td>Normal uterus: 15%</td>
</tr>
<tr>
<td></td>
<td>Secondary amenorrheic uterus: 7%</td>
</tr>
<tr>
<td></td>
<td>Primary amenorrheic uterus: 1%</td>
</tr>
<tr>
<td>Withdrawal-bleeding test</td>
<td>Normal uterus: 14%</td>
</tr>
<tr>
<td></td>
<td>Secondary amenorrheic uterus: 5%</td>
</tr>
<tr>
<td></td>
<td>Primary amenorrheic uterus: 2%</td>
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</tbody>
</table>

The withdrawal-bleeding test has been performed for clinical diagnosis of amenorrhea of uterine origin. However, the numerical figure of the withdrawal-bleeding index has been based upon the extent of the bleeding depending on a subjective estimation and does not always reflect the uterine function as shown in Figs. 11 and 12. The author devised new methods of a laboratory test for the uterus-sensitivity.

1) Uterus-sensitivity test by 32P-uptake through endometrium

Many investigators have reported that the endometrial phosphate metabolism was accelerated by the administration of estrogen in experimental animals. In the present study, the value of 32P-uptake curve can be considered to represent the level of phosphate metabolism of human endometrium. The measurement of the level of the phosphate metabolism using 32P showed that the level was high in the normal uterus, but in the subjects of senile, primary amenorrheic and hypoplastic uteri and in some cases of secondary amenorrheic uteri, it was always lower than in normal uteri. This finding leads to the conclusion that the general metabolism is depressed in the endometrium of amenorrheic,
2) Uterus-sensitivity test by measurement of oxygen consumption

There are many reports, which showed the fluctuation of $Q_o$$^2$-value with the menstrual cycle. In vitro, estrogen is considered to accelerate and progesterone to depress the $Q_o$$^2$-value of human endometrium. In the present study, the change of $Q_o$$^2$-value of endometrium and myometrium by the administration of estrogen and progesterone was expected to be an indicator of uterus-sensitivity to sex hormones.

It must be evidence for the normosensitivity to sex hormones of the uterus with a normal menstrual cycle that its $Q_o$$^2$-value is high and elevated by the administration of sex hormones, and that the $Q_o$$^2$-value in primary amenorrheic and some of secondary amenorrheic uterus is lower and not elevated must represent their hyposensitivity to the sex hormones.

In the case of uterine muscle tissue, the $Q_o$$^2$-value after the administration of estrogen was notably high in the fibroid uterus which is assumed to be accelerated in its general metabolism, and lower in the senile and hypoplastic uterus which is supposed to be depressed in its general metabolism. These results prove this test to be reasonable as a laboratory test of the sensitivity of uterus muscle.

3) Uterus-sensitivity test by histological examination of endometrium

It is well known that endometrium changes its histological picture according to the cyclic changes of ovary, and conversely, endometrial dating from its histological picture is fairly accurate comparing with B.B.T. and menstruation.

In the present study, the presence of histological changes showing progestational picture of endometrium, as examined seven days after administration of estrogen and progesterone, was used as the indicator of the uterus-sensitivity.

The percentage of the uteri which showed the progestational picture was a little higher in the normal uterus than in the amenorrheic uterus, but all of the normal uteri did not always represent the histological change. This test needs more improvement in the dosage of sex hormones.

4) Uterus-sensitivity test by the crystallization of cervical mucus

Crystallization phenomenon of cervical mucus was first demonstrated by Papanicolau in the dried smear of cervical mucus, which was confirmed to be strengthened by the administration of estrogen and weakened by progesterone by Bergman, Campos da Paz, and others.

The author attempted to employ the crystallization phenomenon of cervical mucus as an indicator of uterus-sensitivity.

In this test, no obvious difference was observed among normal, secondary and primary amenorrheic uteri in the ratio of normosensitive and hyposensitive uterus. The result suggests that anomalous sensitivity of cervical endometrium has no correlation with the length of the duration of amenorrhea.
SUMMARY

In order to elucidate the problem of uterus-sensitivity to ovarian hormones, the following experiments were carried out.

1) The author proposed a new disease "Hyposensitive uterus", provisionally denominated, and three cases of it were reported. This disease is characterized by irresponsiveness of the uterus to ovarian hormones, regardless of the normal function of ovary, confirmed by typical biphasic B.B.T. curve, normal value of estrogen and pregnanediol excretion in the urine and confirmation of corpus luteum by laparotomy.

2) Various kinds of methods of laboratory testing of uterus-sensitivity were devised to make diagnosis of anomalous sensitivity of uterus. A summation of data examined by these tests leads to the conclusion that uterus-sensitivity test by the measurement of oxygen consumption of endometrium and myometrium was most convenient and valuable in clinical use.

References