HUMAN SERUM LUTEINIZING HORMONE (LH) LEVELS DURING THE NORMAL MENSTRUAL CYCLE AND AFTER OVARECTOMY*

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The reports on the fluctuation of Luteinizing Hormone (LH) in human urine are scarce, but the reports on the fluctuation in human blood are even more scarce. In our studies described in the former reports, it was demonstrated that the ovarian ascorbic acid depletion method (OAAD, Parlow, 1958) excels in specificity, sensitivity and precision for LH assay. Therefore, we attempted to study, by using this method, the fluctuation of LH in serums of women with normal menstrual cycle and of ovariectomized women.

MATERIALS AND METHODS

Normal menstrual women

Subjects of examination were 19 women who were confirmed by basal body temperature (BBT) and premenstrual endometrial biopsy to have normal ovulatory menstrual cycle. Pelvic examination found no abnormality in them. Their ages ranged from 23 to 35. They were all married. As an index of their estrogenic activity, the quantity of their cervical mucus was measured.

Ovariectomized women

Ten ovariectomized women—9 operated for cervical cancer and 1 for uterine sarcoma—were examined. LH assay was made 10 to 102 days after the operation.

Serum collection

Twenty ml of blood was drawn into sterilized test tubes and left in refrigerator until clotting. Then it was centrifuged at 3,000 r.p.m. for 20 mins. The serum was kept frozen for less than (GCG, "Gonotropin") a week prior to use.

Bioassay

For LH assay, the ovarian ascorbic acid depletion method (OAAD) of Parlow was used, as previously described in detail (Yokota *et al.*, 1965). Immature female rats, 25 to 28 days old, of Wistar-Imamichi or Holtzman strains were pretreated with gonadotrophins as follows: they were first injected subcutaneously (s.c.) with pregnant mare's serum gonadotrophin (PMS, "Sero-tropin") 75 i.u.; 56 to 64 hrs. later they were injected s.c. with human chorionic gonadotrophin (HCG, "Gonotropin") 30 i.u. Six to nine days thereafter they were used for experiments.

Received for publication April 13, 1965.

* This work was partially supported by a grant No. M-65, 18 from the Population Council, New York.

Under ether anesthesia, the left ovaries were removed for analysis of ascorbic acid concentration. Human serum (1~2 ml) was then injected intravenously (i.v.) into jugular vein over a period of 1 min. Two hrs. thereafter in the case of Wistar-Imamichi strain, and 3 hrs. thereafter in the case of Holtzman strain, the right ovaries were removed for analysis of ascorbic acid concentration. Ascorbic acid concentration was determined by a modification of Mindlin and Butler's method (1937). The percentage depletion in ascorbic acid concentration in the second gland as compared with that in the first constitutes the response.

When the number of the animals was sufficiently great, the 3 point assay was used in the design and analysis of OAAD assays *i.e.*, two doses of the reference preparation, NIH-LH, and unknown materials were simultaneously assayed. When the number of the animals was small, the standard dose response curve was used. Four to five animals were used in one assay. One ml of serum of women in preovulatory phase and of castrated women was injected into a animal, and 2 ml of other serum was injected into it. Further, there were few instances when the injected serum acted toxically and killed the animals. LH level in serum was expressed in equivalents NIH-LH $\mu\text{g}/100\text{ ml}$.

RESULTS

1. Parallel assay

Four point assay with two dose levels of the standard preparation (NIH-LH-S₁ 0.5 μg and 2.0 μg) and of the human serum of preovulatory phase (0.5 ml and 2.0 ml), respectively, were employed (Fig. 1). Parallelism was demonstrated between the standard preparation and an unknown preparation at 1 % level of significance. The slope of reference preparation was 40.0, and the slope of serum was 37.5. This shows that the OAAD method can measure serum LH.

Fig. 1. A typical 4-point assay

2. Effect of serum on response to NIH-LH-S₁

In order to check the possibility that human serum may augment LH activity in the OAAD assay, NIH-LH-S₁ 2.0 μg dissolved in 1 ml of saline and 1 ml of the serum in the late luteal phase were injected. i.v. to recipient animals and

OAAD responses were compared with each other. The former gave 31.1 ± 2.9 % OAAD and the latter 33.1 ± 3.2 % OAAD. The difference between them was statistically not significant ($P > 0.1$). In other words, human serum failed to augment the potency of NIH-LH-S₁ in the OAAD assay.

3. Serum LH levels in the normal menstrual women

Individual patterns: It was difficult to draw blood every day throughout a menstrual cycle, so serum LH levels were measured three or four times during one menstrual cycle from 7 normal menstrual women. The results are shown in Figures 2 and 3. It was found that serum LH level was higher in the preovulatory phase than in the follicular and the luteal phase. The fluctuation of the quantity of the cervical mucus, measured as an index of estrogenic activity, and serum LH level corresponded with each other fairly well. Case 7 passed into pregnancy during the examination. The comparatively high level of serum LH activity after the rise of BBT may be considered to show the influence of HCG.

Composite pattern: Figure 4 shows the results of 38 assays on 19 normal menstrual women put together. We considered the day just before the rise of BBT as the center day of the ovulatory phase and expressed it by "0". Serum LH level gradually rose between the follicular phase and the preovulatory phase and reached the peak from "-2" day to "0" day, and after rises of BBT, it rapidly fell.

4. Serum LH levels in ovariectomized women

Serum LH levels of 9 women with cervical cancer and 1 woman with uterine sarcoma were measured after ovariectomy. They were operated 10 to 102 days before LH assay (Table 1). As a whole, the results showed high values but there was a considerable irregularity. Young women showed comparatively high values, sometimes as high as that in the preovulatory phase, whereas old women tended to show comparatively low values.

5. Comparison of serum LH levels in several phases of normal menstrual cycles and in ovariectomized women

As shown in Figure 4, the normal menstrual cycle was divided into five phases: the early follicular phase (-14~-10), the late follicular phase (-9~-4), the preovulatory phase (-3~0), the early luteal phase (+1~+6), and the late luteal phase (+7~+14). Serum LH levels in several phases of normal menstrual cycles and in ovariectomized women were statistically compared with one another. (Table 2).

When Student's t-test was used, serum LH level of the preovulatory phase proved to be significantly higher than that of any other phase, and even than that of ovariectomized women. Serum LH level after ovariectomy was significantly higher than that of any phase of the normal menstrual cycle except the preovulatory and the late follicular phase. The difference in serum LH level between ovariectomized women and the late follicular phase of normal women was not significant. The difference between the early follicular and the late follicular phase was not significant. Further, LH level of the early luteal phase was significantly higher than that of the late luteal phase. Serum LH level was

Fig. 2. Variation of serum LH level during the normal menstrual cycle—individual patterns

Fig. 4. Variation of serum LH level during the normal menstrual cycle—composite pattern

Table 1. Serum LH levels in ovariectomized women

	Case	Age (yrs.)	Diagnosis	Menstrual cycle before ovariectomy	Days after ovariectomy	Ascorbic acid depletion (% $\pm$ S.E.)	Serum LH (equiv. NIH-LH μ g/100 ml)
1	S.K.	44	Cervical cancer	regular	25	23.6 $\pm$ 6.4	132
2	K.Y.	45	do	irregular	32	13.5 $\pm$ 6.8	72
3	K.K.	34	do	regular	52	19.7 $\pm$ 4.6	103
4	T.Y.	32	do	regular	38	18.0 $\pm$ 5.6	92
5	T.N.	35	do	regular	102	13.1 $\pm$ 5.5	68
6	H.O.	59	do	Menopause in 48 yrs.	10	12.5 $\pm$ 4.2	66
7	K.N.	29	do	regular	32	23.4 $\pm$ 4.5	132
8	H.H.	32	do	regular	74	18.5 $\pm$ 6.3	95
9	T.S.	63	do	Menopause in 47 yrs.	35	13.7 $\pm$ 5.1	70
10	S.K.	47	Uterine sarcoma	regular	28	12.5 $\pm$ 4.8	66

Table 2. Comparison of serum LH levels in several phases of normal menstrual cycle and in ovariectomized women

		No.	Mean of serum LH (equiv. NIH-LH μ g/100 ml $\pm$ S.E.)	Range
I	early follicular phase	5	43.2 $\pm$ 4.3	31 ~ 56
II	late follicular phase	8	67.3 $\pm$ 8.4	26 ~ 98
III	pre-ovulatory phase	13	119.8 $\pm$ 9.3	87 ~ 216
IV	early luteal phase	5	55.6 $\pm$ 7.1	36 ~ 72
V	late luteal phase	7	39.0 $\pm$ 2.1	29 ~ 44
VI	after ovariectomy	10	89.6 $\pm$ 8.1	66 ~ 132

p values

highly significant:

- (1) between III and I, II, IV, V ($p < 0.001$)
- (2) between VI and I ($0.001 < p < 0.01$)
- (3) between VI and V ($p < 0.001$)

not significant:

- (1) between I and II ($0.2 < p < 0.3$)
- (2) between VI and II ($0.05 < p < 0.1$)

significant:

- (1) between IV and V ($0.02 < p < 0.05$)
- (2) between III and VI ($0.02 < p < 0.05$)
- (3) between VI and IV ($0.01 < p < 0.02$)

the lowest in the late luteal phase.

DISCUSSION

Reports on the measurement of pituitary gonadotrophins in human blood are extremely small in number. In order to ascertain the real change in hormone secretion, it is more desirable to measure it in blood than in urine in which it

may be in metabolite form. Perhaps, the reason is found in that it is difficult to take blood in a large quantity and that there is no highly sensitive method of assay.

Fluhmann (1929) discovered for the first time the existence of pituitary gonadotrophins in human blood. Freed (1935) reported that he found the luteinizing activity of male and female serum by using the synergistic effect of sheep pituitary extracts.

Since then, no reports on the pituitary gonadotrophins in human blood have been published for some time. But McArthur *et al.* (1956) and Antoniadou *et al.* (1957) proved, by using the uterine weight and ovarian weight method in immature female rats, the existence of gonadotrophic activity in the protein fractions, which were extracted from the pooled human normal and postmenopausal plasma with ethanol or ethanol and zinc salts. And they reported that the normal plasma contained one-eighth of gonadotrophins of pooled postmenopausal plasma.

Further, Apostolakis (1960) proved, by using the mouse uterus test, the existence of gonadotrophic activity in the acetone extracts of human postmenopausal plasma. For the first time he expressed the results in terms of a reference standard (HMG-20A), and measured them with individual plasma specimens. He also examined menstrual women and showed the existence of positive examples only in an approximate mid-cycle. He reported that FSH activity was detected by the "HCG augmentation" test (Steelman and Pohley, 1953) and LH activity by the hypophysectomized rat prostate (H.R.P.) test (Greep *et al.*, 1942) both in the pooled human plasma.

Recently, McArthur *et al.* (1964) succeeded in partial separation of FSH and LH by fractionating the pooled human menopausal plasma with Cohn method 6 and 9 (Cohn *et al.*, 1944).

All of the above reports only proved the existence of gonadotrophins in blood, and there were no reports on the fluctuation of LH in the blood of normal menstrual women who were ascertained to have ovulation.

In this study, we found it possible to measure, by the OAAD method, LH level in the non-pooled, non-extracted human serum, using NIH-LH (ovine) as the reference standard.

When 4 point assay on NIH-LH-S₁ and the human serum (preovulatory phase) was employed, a parallelism was confirmed, and it was found that the human serum had no augmentation effect on NIH-LH. Parlow (1963) described that the prostate assay was influenced by rat and human serum, but the OAAD assay was not influenced by rat serum. He did not, however, describe about human serum.

Thirty-eight assays on 19 normal menstrual women, who had been proved by BBT and endometrial biopsy to have ovulation, were performed. As for fluctuation in serum LH level during the normal menstrual cycle, an upward convexity was formed as above described with the preovulatory level as its peak, which is 87 to 216 equiv. NIH-LH $\mu\text{g}/100\text{ ml}$. This change closely agrees with the fluctuation of LH level in urine reported by McArthur *et al.* (1958a and b). Morat6-Manaro *et al.* (1960) showed the existence of the peak of LH in the ovulatory phase and Taymor (1961) showed the predominance of LH in urine in the mid-cycle. Wide and Gemzell (1962), who measured LH level in urine by the im-

munological method, showed the existence of the peak in the mid-cycle, and described that it was undetectable during menses. Fukushima *et al.* (1964), using for the first time the OAAD method, clarified the fluctuation of LH level in urine during the normal menstrual cycle, and showed that it reached its peak 1 to 3 days before BBT shift. In this way, concerning the fluctuation of LH level in urine, there is a consensus of opinion among the investigators.

On the other hand, Ramirez and McCann (1964) measured, by using the OAAD method, the plasma LH content of the estrus cycle of the rat and found that it reached the peak in the afternoon of proestrus.

From the above mentioned results, it is known that before ovulation, LH is discharged from the pituitary into blood in a large quantity. One of 38 assays showed a very high value, amounting to 216 equiv. NIH-LH $\mu\text{g}/100\text{ ml}$ and perhaps it may have just coincided with the critical point.

Next, serum LH level of 10 ovariectomized women ranged 66 to 132 equiv. NIH-LH $\mu\text{g}/100\text{ ml}$, and this was significantly lower than in the ovulatory phase and somewhat but not significantly higher than that in the late follicular phase. But serum LH level of ovariectomized women had much irregularity, and it was interesting to find that the level of younger women was higher, sometimes as high as that of the preovulatory phase. According to Albert *et al.* (1961), it has hitherto been said that after ovariectomy the secretion of FSH from the pituitary increases chiefly, but our study made it clear that the secretion of LH also increases. This result is similar to those of Ramirez and McCann (1963) who, by using the OAAD method, proved that when female rats are castrated, their plasma LH contents increases.

As regards the metabolism of LH, there are still many indistinct points, but Parlow (1964) described that the ovine LH injected i.v. into rats was excreted in urine to the extent of only 0.4 %. If the same can be said of the human, it may be far better to measure LH level in blood than in urine.

Parlow (1961), Schmidt-Elmendorff and Loraine (1962), and Hutchinson and Worden (1964) described that, in measuring LH level in urine by using the OAAD method, the toxicity after the intravenous injection raises a question. We also recognized that in some instances animals died after intravenous injection of some urinary extracts. In this respect, human serum had no toxic effect on rats.

SUMMARY

1. Human serum LH levels were assayed by using ovarian ascorbic acid depletion method. The 4 point assay using 2 doses of NIH-LH-S₁ and of human serum demonstrated parallelism. Human serum failed to augment LH activity in ovarian ascorbic acid depletion test.

2. The human serum LH level of normal menstrual women showed the peak in the preovulatory phase. Ovariectomy in women considerably increased serum LH level.

ACKNOWLEDGEMENTS

NIH-LH was kindly provided by Dr. Wilhelmi of National Institutes of Health.

PMS (Serotropin) and HCG (Gonotropin) were kindly provided by Teikoku Hormone Mfg. Co.

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