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Variations in the Maternal Serum Prolactin Levels during Pregnancy, Labor, and the Puerperium

(serum prolactin/pregnant women/lactation)

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An estimation of the blood HPr levels in normal nonpregnant women, pregnant women, and puerperal women by the RIA method was carried out. The results were as follows.

1) Serum HPr of normal nonpregnant women 30 min after at rest at 10 a. m. stood at 11.42±3.38 ng/ml.

2) Serum HPr in normal pregnant women showed a slightly higher level during the early stage of pregnancy compared with normal nonpregnant women, and it increased gradually to about a 7-fold increase during the terminal stage of pregnancy.

3) Serum HPr level during labor increased in some cases and decreased in others, showing no fixed tendency.

4) Serum HPr during the puerperium showed the highest level on the 3rd day of the puerperium, and then gradually decreased to show a level close to that of normal nonpregnant women on the 30th day of the puerperium.

5) Regarding the variations in serum HPr before and after breast massage in normal nonpregnant women, pregnant women and puerperal women, the HPr levels showed significant increases after breast massage and the increase rate was the highest in women on the 5th day of the puerperium.

6) In puerperal women on the 1st and 7th day of the puerperium showing high levels in serum HPr, diurnal variations in their HPr were observed as in nonpregnant normal women ; the HPr levels tended to be higher at night than at daytime, showing the increase rates of about 100 to 400 per cent.

Since human prolactin (HPr) was separated as an independent hormone from the human pituitary body and the radioimmunoassay (RIA) of HPr was developed by Friesen *et al.*(1), new findings on the mode of its secretion have come to be obtained in the domains of obstetrics and gynecology as well.

HPr is a peptide hormone which is produced from the acidophilic α cells in the anterior lobe of the pituitary body and whose molecular weight is 22,000 to 35,000, its being made up of 198 amino acids(2).

Its physiological action is known to differ considerably according to the animal species.

Its physiological significance in man having gradually become clear, HPr is said to play an important role in the growth of the mammary gland, lactation, and pregnancy. As to the significance of the variations in HPr, however, there still are many points in which require clarification.

In their investigation of these variations in the maternal blood levels of HPr during pregnancy, labor, and the puerperium, the authors measured these levels by the RIA method during each period, the results of which are reported here as part I.

EXPERIMENTAL PROCEDURE

Materials

The materials for our comparative study on serum HPr during pregnancy, labor, and the puerperium consisted of blood samples taken from the pregnant and puerperal out-and in-patients seen at our department of obstetrics and gynecology from July to October, 1976.

(1) During pregnancy, the samples were collected at 10 a. m. from 42 pregnant out-patients.

(2) During labor, the blood samples were collected on three occasions, namely, on admission with labor pain, immediately after delivery, and 3 hr after delivery from nine pregnant women in hospital ; and, during the puerperium, the blood samples were collected at 10 a. m. on the lst, 3rd, 5th, and 30th days.

(3) For the effects of breast massage, the blood samples were collected on two occasions, namely, at 10 a. m. just before massage and 30 min after massage from a total of 22 women consisting of normal non-pregnant outand in-patients and of puerperal women on the lst, 3rd, 5th, and 30th days of the puerperium.

(4) For the measurement of diurnal variations in serum HPr, the blood samples were collected every 4 hr begining at 10 a. m. for 24 hr from each of nonpregnant women and puerperal women on the lst and 5th days of the puerperium.

Assay for Prolactin

(1) Measurements of blood HPr were carried out in duplicate with CIS-PROLAK-KIT (Midorijuji radioisotope laboratory) using ^{125}I-labelled prolactin(3). The assay procedure is outlined in Table I.

(2) To see the reproducibility, six plasma samples of an identical pregnant woman were measured for HPr. The reproducibility averaged 17.08 ± 1.08 ng/ml as shown in Table II, the coefficient of variations being relatively good at 6.3%.

(3) Recovery of prolactin added. When 12.5, 25.50 and 100 ng/ml of prolactin were added to the plasma of normal nonpregnant women, the recovery was calculated to be 119.88 ± 9.51 per cent on the average (Table III).

RESULTS

1. Serum HPr Level in Nonpregnant Normal Women

TABLE I. *The Procedure for the Radioimmunoassay of Prolactin*

Step	Group No. of test tubes			
	1–2 total activity	3–4 blank	5–18 standard	19–100 sample
Buffer	—	0.15 ml	0.05 ml	0.1 ml
Standard	—	—	0.1 ml	—
Sample	—	—	—	0.05 ml
^{125}I-Prolactin	0.1 ml	0.1 ml	0.1 ml	0.1 ml
Antiserum	—	0.1 ml	0.1 ml	0.1 ml
Mix	mixed by vortex mixer			
Incubation	40–44 hrs at room temp.			
Immunosorbant		0.5 ml	0.5 ml	0.5 ml
Incubation	Under stirring for 4–6 hr. at room temp.			
Centrifuge	2500 g for 10 min at room temp			
Diluted buffer	—	2 ml	2 ml	2 ml
Wash	Mixed → centrifuge → decant			
Count	Well type scintillation counter			

TABLE II. *Reproducibility of Serum Prolactin Radioimmunoassay*

No.	Measurement (ng/ml)
1	16.0
2	17.5
3	16.5
4	18.5
5	16.0
6	18.0

The serum obtained from a woman in 8 weeks of pregnancy. Mean value±SD=17.08 ±1.08. Coefficient of variation=6.3%.

TABLE III. *The Recovery Rate of Prolactin*

No.	Additional	Recovered	Recovery
	ng/ml	ng/ml	%
1	12.5	16.3	130.5
2	25.0	31.25	125.0
3	50.0	57.0	114.0
4	100.0	110.0	110.0

Average of recovery rate = 119.8±9.5%.

The HPr value 30 min after rest at 10 a. m. averaged 11.42±3.38 ng/ml in five normal women (20 to 36 yr of age) whoes menstrual cycle fell on the luteal phase (Table IV).

2. *Serum HPr Level in Normal Pregnant Women*

The mean values of the serum HPr in normal pregnant women at 8–42

TABLE IV. *Serum Prolactin Concentration in Normal Nonpregnant Women*

No.	Serum prolactin (ng/ml)
1	14.4
2	11.6
3	9.6
4	10.5
5	11.0

Mean value±SD=11.4±3.3.

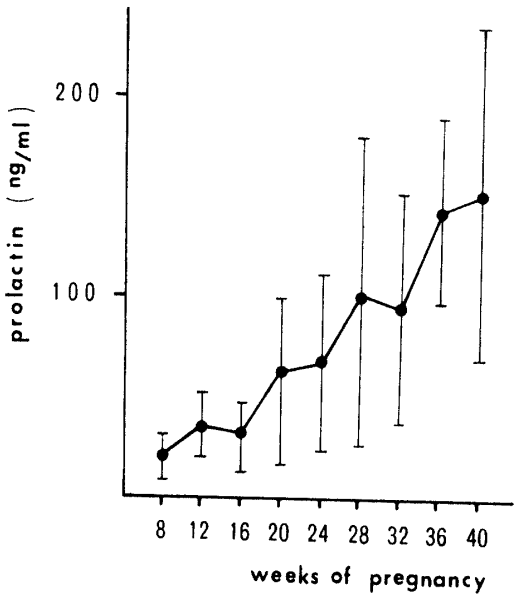

Fig. 1. Changes in maternal serum prolactin concentration throughout weeks of pregnancy.

weeks' gestation were calculated every four weeks (Fig. 1).

At 8 weeks' gestation, serum HPr value stood at 20.8±10.8 ng/ml, already slightly higher than that in nonpregnant women. Thereafter, it gradually increased with some variations to reach 102.3±77.9 ng/ml at 28 weeks' gestation and 152±83.6 ng/ml at 40 weeks' gestation, which is about seven times that of the early stage of pregnancy.

3. Serum HPr Levels in Labor and the Puerperium

The mean values of maternal serum HPr were found on admission with labor, immediately after delivery, three hr after delivery, and on the lst, 5th, and 30th days of the puerperium in nine normal women (Fig. 2).

The HPr value was 254.3±159 ng/ml on admission and it tended to decline slightly to 167.5±70.5 ng/ml immediately after delivery.

Three hr after delivery, it varied widely at 277.8±272 ng/ml on the average, abnormally high values being observed in some cases.

On the 3rd day of the puerperium, the value was still high at 263±171 ng/ml. This was followed by a significant decline ($p<0.05$) which reached 146.2 ±108.1 ng/ml on the 5th day and 19.6±12 ng/ml on the 30 th day of the puerperium.

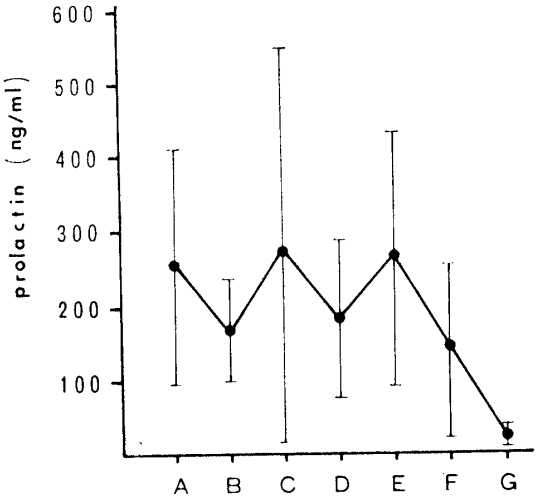

Fig. 2. Maternal serum prolactin concentration during labor and the puerperal period.
A : time of admission with labor pain, B : immediately following birth of the child, C : 3 hr after infant birth, D : the first day, E : the 3rd day, F : the 5th day, G : the 30th day of the puerperium.

4. Variations in Serum HPr before and after Breast Massage in Normal Nonpregnant Women and in Puerperal Women

The increase rates in HPr by breast massage were expressed with the value before massage as 100 in 8 normal nonpregnant women and 14 normal puerperal women on the 3rd, 5th, and 30th days of the puerperium (Fig. 3).

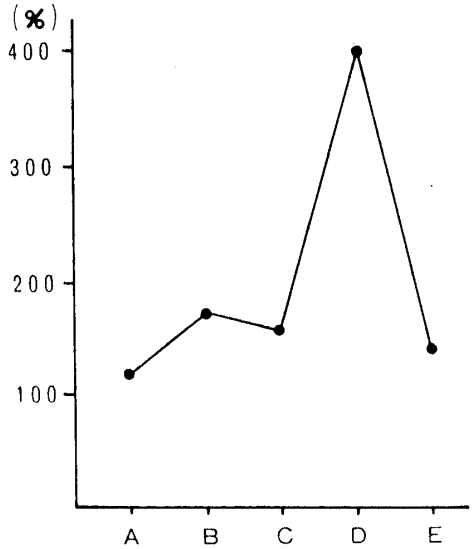

Fig. 3. The increase rates in serum prolactin concentration in nonpregnant women and puerperal women before and after being their breasts have been stimulated by massage.
A : normal nonpregnant women, B : puerperal women on the first day, C : on the 3rd day, D : on the 5th day, E : on the 30th day.

After breast massage, the mean HPr level was 118% in normal nonpregnant

women. In puerperal women, it was 169% on the lst day, 148% on the 3rd day, 407% on the 5th day, and 137% on the 30th day of the puerperium, a significant increase rate ($P<0.05$) being seen in all cases.

On the 5th day of the puerperium, the increase rate was reached to 407 per cent despite the tendency of its HPr level to decline from the peaks of 3rd day. On the 30th day of the puerperium, however, it stood at 137 per cent, showing no significant difference statistically from non-pregnant women ($P<0.5$).

5. *Diurnal Variations in Serum HPr*

Serum HPr was measured on a time-course basis every four hr in normal non-pregnant women and in puerperal women on the lst and 7th days of the puerperium (Fig. 4).

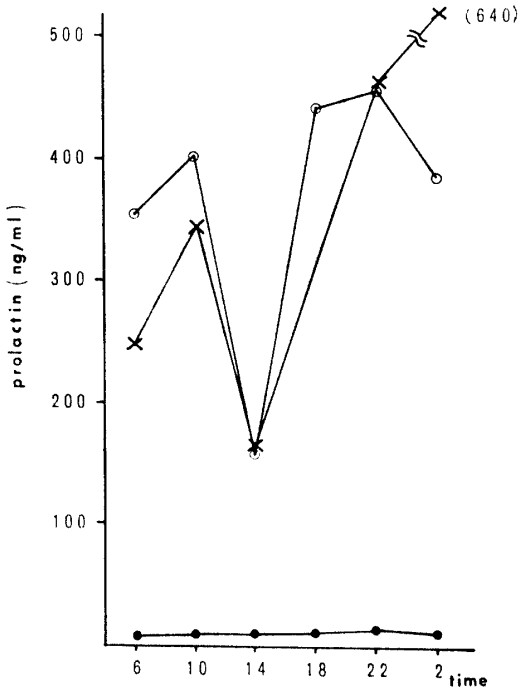

Fig. 4. Diurnal variations in serum prolactin concentration in puerperal women and normal nonpregnant women.
●——● : nonpregnant women, ×——× : puerperal women on the first day, ○——○ : puerperal women on the 7th day.

Diurnal variations in the HPr levels in normal nonpregnant women ranged from 7.6 ng/ml to 15.2 ng/ml ; these levels tended to be higher at night, showing increases of approx. 50 to 90 per cent.

In women on the lst day of the puerperium, despite the high HPr levels, the diurnal variations ranged from 160 to 640 ng/ml ; the levels tended to be particularly higher at night, showing increase rates of approx. 180 to 300 per cent (Fig. 4).

On the 7th day of the puerperium, the diurnal variations ranged from 68 to 440 ng/ml ; the values tended to increase at night and the increase rate of approx. 100—400 per cent was noted (Fig. 4).

DISCUSSION

To investigate the serum HPr levels in normal nonpregnant women and in normal women during pregnancy, labor, and the puerperium, we measured them with HPr-RIA-KIT.

The sensitivity of measurement and the results obtained in the recovery and reproducibility tests in the fundamental experiments confirmed the validity of the assay method described here.

In making measurement, the subject of measurement often showed high values of HPr in the field of obstetrics and gynecology. So, HPr was diluted with phosphate buffer solution ; the recovery rate of the 4-fold dilution thereof was 102 per cent. Therefore, values measured in the present experiment are considered reliable.

According to results of the present experiment, the serum level of HPr in the luteal phase was 11.42 ± 3.38 ng/ml which is similar to values hitherto reported, that is, 10.27 ± 7.49 ng/ml by Frantz *et al.*(4) and 13.10 ± 1.96 ng/ml by Wada *et al.* (5).

When maternal serum HPr levels were studied at 4 weeks intervals during the course of gestation in normal pregnant women, it reached 20.8 ± 10.8 ng/ml at weeks' gestation, significantly higher than that of normal nonpregnant women, it thereafter increased gradually with the advance of the number of pregnant weeks to become about 11 times higher at 40 weeks' gestation than that of normal nonpregnant women or about seven times higher than that in the early stage of gestation.

Shoji *et al.*(6) rendered a similar report, with the serum HPr levels being 22.4 ± 13.4 ng/ml at 8 weeks' gestation. According to the report of Kato *et al.*(7), it was 43.0 ± 11.7 ng/ml during the early stage of pregnancy and 231 ± 70.5 ng/ml during the terminal stage of pregnancy.

Regarding variations in HPr during pregnancy Kurozumi(8) maintained that they are influenced either by variations of estrogen during pregnancy or morphological changes in the HPr-producing cells brought about by estrogen. Variation in HPr levels via TRH has also been taken up for discussion. On the basis of the fact that the HPr level rises following an administration of estradiol, Yen *et al.*(9) pointed out a positive feedback in the relation between HPr and estrogen (10). Berberia *et al.* also reported that estradiol and HPr show similar fluctuations with the increase in the number of pregnant weeks.

From variations of estrogen during pregnancy, Masada(11) inferred that the rise in HPr levels might possibly depend on estrogen. TRH increases the secretion of TSH as well as that of HPr(12), and estrogen is said to accentuate the reaction of HPr to TRH(13). Therefore, it is surmised that an increase in estrogen will be concerned with variations in HPr during pregnancy directly or indirectly via TRH.

The diencephalon and pituitary in labor appear to be under a strong stress as can be seen from the variations in maternal blood ACTH(14). According to Frants(4), the blood HPr levels rise under various types of stress and in exercise. In the present experiment, however, the variations of HPr during

labor showed no fixed tendency ; the HPr values were uneven with a tendency to decline slightly at delivery compared with the time of labor onset with some cases showing high values and others low values 3 hr after childbirth.

When various placental hormones would fall off sharply after labor, the HPr showed the highest level ever recorded throughout pregnancy, labor and puerperium on the 3rd day of the puerperium. Then it decreased significantly on the 5th day ($P<0.05$) to reach the early pregnancy level on the 30th day of the puerperium.

The results of the present experiment showing a peak of HPr on the 3rd day of the puerperium are similar to those reported by Hoshiai *et al.*(15) showing a peak at 40 weeks' gestation except for the difference in time of peak.

Breast massaging at lactation during the puerperium is said to accelerate secretion of HPr. When studies were made on the variations in HPr induced by breast massaging and the difference in reactivity between normal nonpregnant women and puerperal women, differences in HPr before and after breast massage were observed in both the normal nonpregnant women and puerperal women. The HPr rose transiently after breast massage and the rate of increase varied from case to case. The rate of increase was particularly high on the 5th day of the puerperium showed the highest rate of increase of HPr ($P<0.05$) despite the tendency to decrease in HPr concentration.

Similar results have also been reported by other investigators measuring HPr before and after sucking stimulation at various times during the puerperium. Tyson *et al.*(16) reported that the HPr level rose from 124 to 163 ng/ml 30 min after the sucking stimulation for one week after delivery, that it was low before sucking but showed about a 6-fold increase after sucking for 10 to 40 days after delivery, and that it showed only slight variations after the sucking stimulation from 80 days after delivery.

Shioji *et al.*(6) reported that the HPr level fell off gradually from 2—3 weeks to 2—3 months after delivery but that it was about twice the level of normal women, that when puerperal women were made to lactate during this period, the HPr level showed a 10 to 20-fold increase within 15 to 30 min, but that the blood HPr level three to four months after delivery returned to the level of normal women and did not increase even after lactation.

Also, there are reports on the influence of drugs on the reaction of HPr to breast massage. Brun *et al.*(17) maintained that the HPr level falls off and an increase in HPr due to breast massage is not observed in cases in which Br-ergocryptone has been administered to puerperal women within seven days after delivery. Gerson *et al.*(18) reported that an increase in HPr due to breast massage was observed even on administration of Br-ergocryptone but that the increase was mild.

As indicated by results of the present study and also by the reports mentioned above, the prolactin-releasing action of breast massage is a certainty. Moreover, there was a difference in the prolactin-releasing action of breast massaging in nonpregnant and puerperal women probably because of the reactivity of the

hypothalamic nucleus to breast massage and releasing factors and suppressing factors of HPr secreted from the hypothalamus being different under various hormonal conditions. Further, it has been surmised that the said action might be decreased or blocked by Br-ergocryptone.

HPr is said to play an important role in the starting and maintenance of lactation, as seen in results of the present study ; however, the HPr level is high during the latter period of pregnancy, a period in which lactation does not occur.

But lactation was noted from the third day of the puerperium in all puerperal women in the present study with labor as a turning point, a time when blood HPr rather tended towards gradual decrease. Accordingly, it appears impossible to account for the initiation or maintenance of lactation with a mere increase in blood HPr.

Referring to recent findings about lactation and maternal blood HPr, Tyson *et al.*(16) attributed the suspension of lactation during gestation to an interruption of lactation by estrogen and progesterone, both of which had become increased due to pregnancy. Assari *et al.*(19) reported that the administration of progesterone to rabbits inhibited the start of lactation.

It is generally believed that estrogen derived from the placenta takes part in the growth of the ductus in the mammary gland and that progesterone is concerned with the growth of its acinus. After performing various kinds of animal experiments, Yokoyama(20) held that a combination of estrogen and progesterone secreted from the placenta during gestation might suppress the secretion of a group of lactation-starting hormones from the anterior lobe of the pituitary body while it also acts on the mammary gland cells to lower their sensitivity to the lactation hormone group. Accordingly, he further explained, a fall in the progesterone/estrogen ratio or a decrease in the two would cause the secretion of a group of lactation-starting hormones from the pituitary body to increase and further enable the mammary gland to respond to the hormone group, which in turn initiates the lactation.

Brun *et al.*(17) having made a detailed study on the relation between blood HPr and lactation, reported that there was no direct correlation between them. However, Aono *et al.*(21) reported that milk secretion was more closely correlated with the increase rate in HPr following nipple stimulation than with the blood HPr level and that puerperal women showing a higher rate of increase in HPr showed a larger secretion of milk.

At any rate, the mechanism concerning the inauguration maintenance of lactation cannot be explained by the results of our experiment.

Judging from a large number of the reports hitherto published, blood HPr seems to play an important role in the start and maintenance of lactation. However, this mechanism is not one that can be accounted for merely by variations in HPr. Besides, there are many other factors, such as placental hormone, ACTH-corticoid series, TSH-thyroxine series, and GH appear to participate in this process in a complicated manner.

Concerning the diurnal variations in serum HPr in normal nonpregnant

women, Kato *et al.*(7) collected blood frequently over 24 hr and their measurements of the HPr levels disclosed minute episodic changes occuring at daytime and large increases taking place during sleep at night. Ehara *et al.*(22) reported that HPr increased with sleep as a turning point.

The results of the present study revealed that the HPr level tended to be higher at night than at daytime, showing an increase of about 50 to 90 per cent, although small episodic changes could not be obtained from the HPr values measured by collection of blood every four hr.

Regarding diurnal variations in HPr in pregnant women, Kumasaka(23) reported that diurnal variations were observed in pregnant women during the early stage of pregnancy as in normal nonpregnant women, that there was a close relationship between HPr secretion and sleep, and that the HPr level increased after sleep.

In the present investigation, no study was made on diurnal variations in pregnant women, but we plan to study them during the terminal stage of pregnancy. On the diurnal variations of HPr in puerperal women, no publications have appeared to date.

According to results of the present study, the HPr level was high on the lst and 7th days of the puerperium compared with that of nonpregnant women, but the puerperal women at both dates showed diurnal variations which showed increases of about 100 to 400 per cent at night somewhat as they did in nonpregnant normal women. Thus, it is surmised that pregnant and puerperal women will show the same functional pattern of the pituitary body of the diencephalon as nonpregnant women do.

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