子宮運動に対する非特異ヒアルロニダーゼ抑制物質の役割について

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Possible Role of the Nonspecific Hyaluronidase Inhibitor in Serum on the Uterine Motility

By

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Biological significance of the nonspecific hyaluronidase inhibitor (NSHI) in serum is obscure. Haas (6) has developed the concept that the serum hyaluronidase inhibitor represents the body defense mechanism against bacterial, and other invasions facilitated by hyaluronidase (H-ase). Hechter and Scully (8), however, indicated that in the *in vivo* environment of skin, the reaction between serum antihyaluronidase and H-ase, either does not occur, or if a reaction does take place, it is readily reversible. Moreover the enzyme added to synovial fluid is not inhibited though this fluid contains all known serum proteins (12, 14).

NSHI concentration in blood serum varies following various physiological and pathological conditions of the animal body. Hakanson and Glick (7) have reported that the H-ase inhibitor level in serum of the woman increased during or just after the labour and reached a peak between the 2nd and 5th postpartum days returning to normal level the 10th day after delivery.

The present experiments are concerned with the changes of NSHI levels in rabbits' blood sera during gestation, after parturition and artificial abortion, and further with a possible role of NSHI on the motility of uterine smooth muscle.

Materials and methods.

A Japanese white breed of rabbit weighing about 3 kg was used through experiments 1 to 4. In experiment 5 Wistar strain female rats bred in our laboratory were used.

For the measurement of NSHI level in serum the viscosimetric technique described by Haas (6) and Glick & Gollan (5) was employed. Experimental details have been given previously for the preparation of the test substances, for the arrangement of the tests and for the calculation of the results (9). Blood samples of rabbits were collected from Saphena veins. The same preparations of enzyme and substrate were used at least throughout one experiment.

Experiments and results.

EXPERIMENT 1. CHANGES OF NSHI LEVELS IN PREGNANT RABBIT'S SERUM.

Each of nine does was bred twice by fertile bucks, six of them have been pregnant

and the other three, failed to become pregnant, were regarded as pseudopregnant, though the persistency of corpus luteum had not been examined. Five unmated does were served as control. NSHI activities were measured at 9th, 18th and 27th day of pregnancy.

The results are presented in Table 1, in which NSHI level in the serum of pregnant

Table 1. Changes of NSHI level in the rabbits' sera during pregnancy.

Situation	No. of rabbits	Days of pregnancy		
		9	18	27
Pregnant	6	11.2±5.64	4.6± 5.97	29.1±4.96
Pseudopregnant	3	3.3±2.25	2.5± 0.70	10.8±5.95
Control	5	5.4±5.90	3.6±10.08	3.7±3.76

rabbits remained almost constant during pregnancy, but showed a marked increase at 27th day of pregnancy. Though there is some increasing tendency at implantation period (9th day of pregnancy), the measurements during 4th to 11th days of pregnancy in 3 other pregnant does did not show any rising. Pseudopregnant rabbits indicate slightly lower values at 9th and 18th day than normal controls, but it is difficult to regard them as the effects of progestational hormones, for these values are within the variance of normal levels.

EXPERIMENT 2. THE EFFECT OF PROGESTERONE ADIMINISTRATION ON NSHI LEVELS IN RABBITS' SERA.

Three castrated male rabbits received intramuscular injection of 3.0 mg of progesterone daily for three days. NSHI levels were measured previous to the experiment and until 120 hours after the first injection. Table 2 exhibits the results. Progesterone treated

Table 2. Effect of progesterone treatment on NSHI level in sera of three castrated male rabbits.

Time passed after the initial injection (hours)	0	6	24	48	72	96	120
NSHI level	3.33 ±2.89	−1.33 ±9.57	−2.30 ±5.21	−3.13 ±5.43	−0.80 ±3.57	−4.00 ±4.10	−1.10 ±4.84

serum seems to accelerate the H-ase activities.

EXPERIMENT 3. CHANGES OF NSHI LEVEL IN THE RABBIT'S SERUM DURING THE PRE- AND POSTPARTUM PERIOD.

NSHI levels of 5 pregnant animals were measured during the one week before and after parturition. The results are showed in Figure 1, NSHI levels begin to increase steeply at 4th-3th day before parturition and reach the peak values the 1st or 2nd day after parturition, returning to normal levels at 5-7th day of postpartum.

EXPERIMENT 4. INFLUENCE OF ARTIFICIAL ABORTION ON NSHI LEVEL IN RABBIT'S SERUM.

Five rabbits were artificially aborted at the 23rd day of gestation when NSHI level is

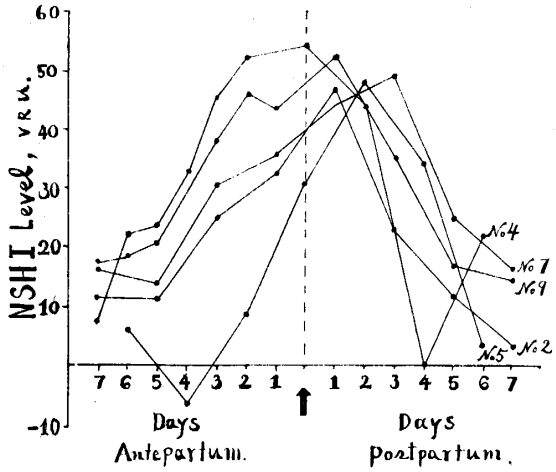

Fig. 1. Changes of NSHI levels in the rabbits' sera at prepartum and postpartum.

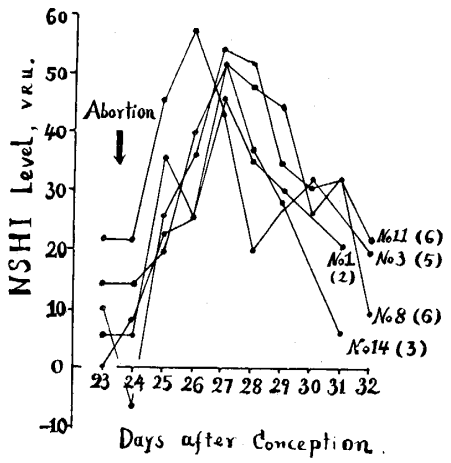

Fig. 2. Changes of NSHI levels in the artificially aborted rabbits' sera.

still low, by injecting 5 ml of saturated saline in each pregnant horns. All of the foetuses were extruded as early as 18 hours. The NSHI levels were assayed daily for 10 days. The results are showed in Figure 2, in which the numeral in brackets denotes the number of foetuses aborted. Some placentae have been extruded on the next morning. NSHI remained on normal levels for one day, followed drastic increase at the 2nd day, and reaching a peak at the 3rd to 4th day and returning to normal values the 8–9th day after the operation.

EXPERIMENT 5. THE EFFECT OF THE SERUM AT VARIOUS CONCENTRATIONS OF NSHI ON THE MOTILITY OF THE ISOLATED RAT'S UTERUS.

Wistar strain rats were used throughout this experiment. Uterine contraction was studied by Magnus method at 37.5°C, suspending the excised rat's uterus in 50 ml of Tyrode's solution. The composition of Tyrode's solution in this experiment is as follows;

NaCl	8.00 g,
KCl	0.20 g,
$CaCl_2$	0.24 g,
$MgCl_2$	0.10 g,
NaH_2PO_4	0.05 g,
$NaHCO_3$	used enough amount to adjust PH at 7.4 (about 0.3 g),
glucose	1.00 g,
redistilled water	1,000 ml.

Uterine segment used is excised from the ovarian portion of the cornu uteri at stage III, and the length of segment is about 20 mm under 5 g stress. The recording on the smoke drum is begun as soon as the tissue has established a typical rhythmic spontaneous contractions. After recording the normal spontaneous contractions for about 10 to 20 minutes as a control, 0.2 ml of test serum is added. The recording is continued for 15 minutes or more after the serum addition, because the effect of serum on the uterine tissue contractions become obvious at 5 to 15 minutes after the addition of serum in most instances. All experiments were finished at the latest within 3 hours after an uterine segment

was seperated from the carcass. The same uterine segment was unable to be used repeatedly, because the influence of the previously added serum remained after washing the tissue.

Table 3. Treatments of the donors for the sera.

Treatments	Time of sampling	Number of rats
1, Adrenalectomized in 2 steps with 4 days interval 7 days after overiectomy, maintained on saline.	4 days after the 2nd operation.	5
2, Hypophysectomized 30 days after orchiectomy, maintained on saline.	20 days after extirpation.	2
3, Ovariectomized.	7 days after operation.	13
4, 0.5 ml of 1.5% formalin solution was subcutaneously injected twice daily for 15 days; 7 days after ovariectomy.	24 hours after the last injection.	5
5, Uterine horns were incised longitudinary to about 2 cm length 7 days after overiectomy.	4 days after operation.	3
6, 0.2 ml of 10% Ferric chloride solution was injected subcutaneously 7 days after overiectomy.	48 hours after injection.	3

To obtain the sera at various degrees of NSHI levels, procedures showed in Table 3 were performed on 29 female and 2 male adult rats. Blood samples were taken by cardiac puncture and after allowing to clot in room temperature for about 30 minutes, the sera seperated by centrifugation. These sera were stored in the refrigerator at 0°C until used. Then NSHI titres were assayed and used for uterine motility. The experiments were finished within 6 hours after the blood was collected, in this period NSHI titres did not change.

Figure 3 shows the uterine responses to 4 serum samples of such degrees of inhibitor levels; A; 62.5 vru, B; 44.8 vru, C; 34.0 vru and D; 20.0 vru per ml of serum respectively. A criterion for the degrees of uterine motility was determined as Table 4 in which A is the average length of ampli-

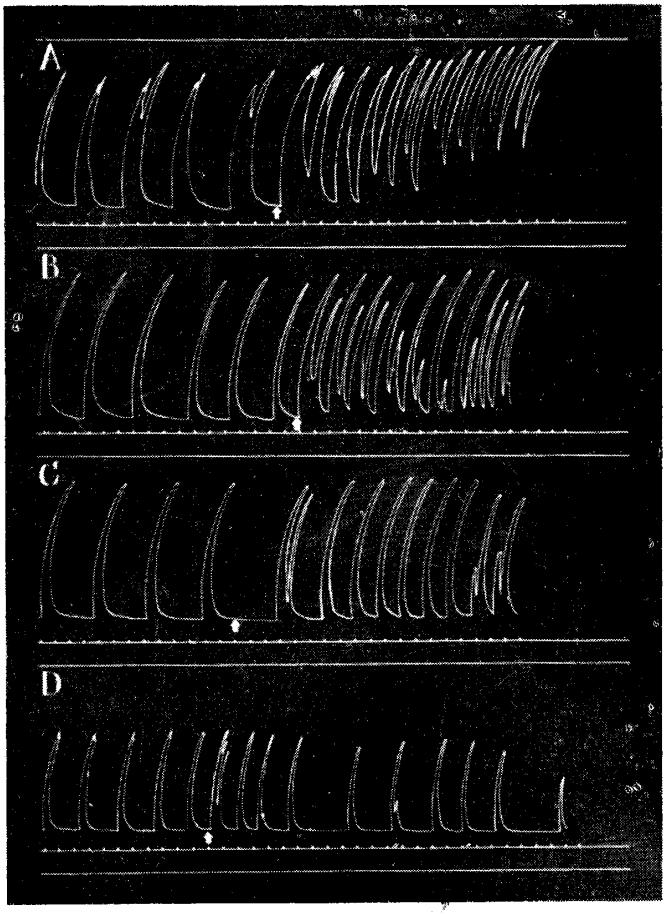

Fig. 3. The responses of the isolated rats' uteri to the sera of various degrees of NSHI.
Inhibitor activity of each serum is A; 62.5 vru, B; 44.8 vru, C; 34.0 vru and D; 20.0 vru per ml of serum respectively. Time, 1 minute.

Table 4. Criterion for the determination of the motility of the rat's uterus to serum.

	−	±	+	++	+++
Rate of contraction	$t > T$	$t = T$	$T > t \geq T/2$	$T/2 > t \geq T/3$	$T/3 > t$
Amplitude	$a \leq 0.9A$	$0.9A < a \leq 1.1A$	$1.1A < a \leq 1.3A$	$1.3A < a \leq 1.5A$	$1.5A < a$
Tonus	—	$h = 0$	$0 < h \leq A/6$	$A/6 < h \leq 2A/6$	$2A/6 \leq h$

T and A: average values on the control.
t and a: average values from 5th to 15th minute after serum.
h: the value at the highest point during 15 minutes after serum.

tude in the control spontaneous contractions, a is average length of amplitude between 5 to 15 minutes after serum addition, T is the average time interval of the neighbouring two contractions in the control, t is the average time interval of the neighbouring two contractions between 5 to 15 minutes after serum addition and h is the height of the base line at the heighest point during 15 minutes after the serum addition. On this criterion, the results of uterine responses to the 31 rats' serum samples are summarized in Table 5. This table shows the significant association between inhibitor levels and degrees of contractions ($P<0.01$).

With 11 serum samples, NSHI was completely inactivated by heating at 60°C for 20 minutes. Six of them had no effect upon the uterine contractility as shown in Figure 4, F, though the same active serum (37.5 vru) severely stimulated uterus as in Figure 4 E. Four inactivated sera, however, inhibited the spontaneous contractions as in Figure 4, H and one serum as in Figure 4, J in spite of the stimulating effect of their active sera; G (34.3 vru) and I (46.0 vru).

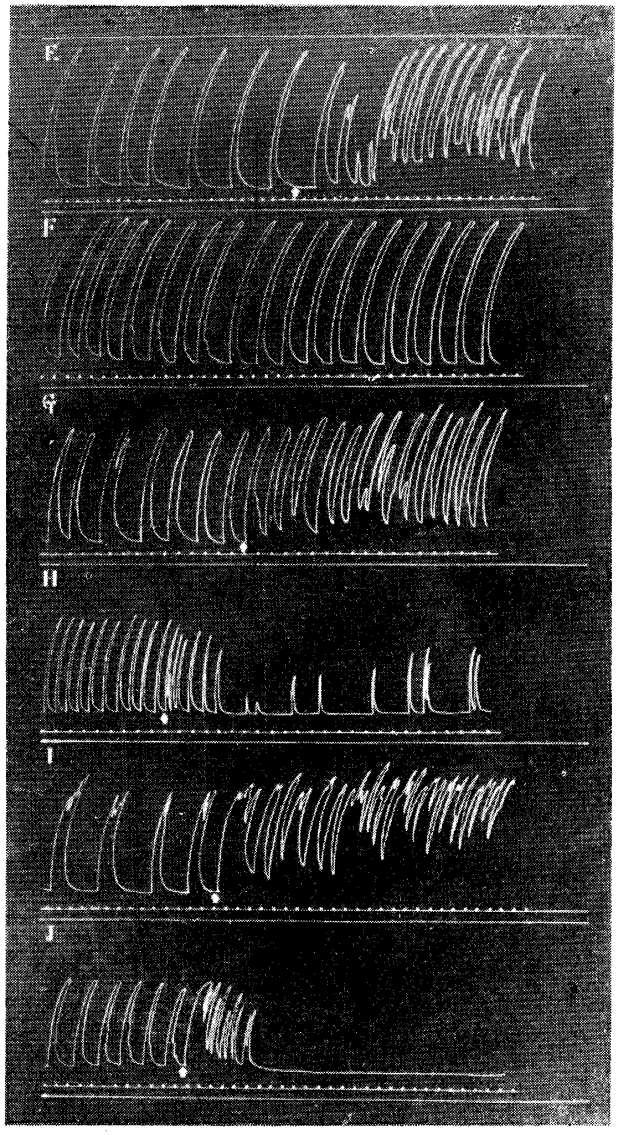

Fig. 4. The responses of the isolated rats' uteri to the NSHI inactivated sera.

The activity of each serum added is E; 37.5 vru, F; serum E inactivated, G; 34.3 vru, H. serum G inactivated, I; 46.0 vru, and J; serum I inactivated. Time, 1 minute.

Degree of contractions / NSHI level	−	±	+	++	+++	++++	+++++	+++++	Total	Hypophysi-ectomy	Adrenal-ectomy	Ovariectomy	Formalin treated	Uterus incised	Ferric chloride
HI ≤ 20	3		1	1					5		5				
20 < HI ≤ 30	1		3	1					5	2		3			
30 < HI ≤ 40			1	2	5	2			10			6		2	2
40 < HI ≤ 50						4	2		6			3	2		1
50 < HI						2	2	1	5			1	3	1	
Total	4	0	5	4	5	8	4	1	31						
Hypophysiectomy			1	1											
Adrenalectomy	3		1	1											
Ovariectomy	1		2	2	3	2	2	1							
Formalin treated						4	1								
Uterus incised					1	1	1								
Ferric chloride			1		1	1									

Table 5. Distribution of sera according to degree of contractions and NSHI level.

Table 6. Distribution of sera according to the degree of contractions and NSHI level when Locke-Ringer solution was used as a physiological solution.

Degree of contractions / NSHI level	±	+	++	+++	++++	Total
20 < HI ≤ 30	1	1	2			4
30 < HI ≤ 40			2	1		3
40 < HI ≤ 50				1		1
50 < HI					2	2
Total	1	1	4	2	2	10

When Ringer-Locke solution was used in place of Tyrode as the physiological solution, the stimulating effect of serum was not so remarkable as in Tyrode's solution. In this instance, tonus was not so affected as in Tyrode, though the frequency was more increased by higher inhibitor serum than by lower. Table 6 shows the results of sera in Ringer-Locke solution.

Preparation of posterior lobe of rat's pituitary by the acetone dehydration was added to Tyrode solution and serum. These solutions did not lose their oxytocic activities by heating at 60°C for 20 minutes.

Discussion.

The H-ase inhibitor levels in sera of the women increase during or just after the labour (7). The present data indicates NSHI level in the rabbit's serum remains almost constant during pregnancy, but it begins to rise at 4 or 3 days prepartum and reaches a peak the first two days after parturition. The cause of the postpartum ircrease of NSHI is not yet clear. But two factors may be responsible for this phenomenon. One is the tissue destruction of placenta and the other is the withdrawal of the depressing effect of progesterone. If the rise of NSHI at parturition is due to these two factors, it may be

supposed that the degeneration of placental tissue begins to increase between the 3rd and 4th days prepartum and that the secretion of progesterone decreases in this period.

In the artificicial abortion NSHI keeps the normal level during the first day. It may be due to the depressing effect of the hypersecreted adrenal cortical hormones as in the reaction phase of the formalin stress previously reported (9).

The normal rabbit's serum sometimes accererates the viscosity reducing ability of H-ase, however the serum itself does not reduce the viscosity of hyaluronic acid solution. This enzyme activation by the serum is recognized by Meyer and Rapport (11) and they ascribe this activation to a nonspecific protein effect.

The increased NSHI level in parturition may be a reflexion of the degeneration of placental connective tissue. But it is not yet clear whether this increased inhibitor has any patho-physiological role or not. It might be a body defence mechanism to protect bacterial invasion at parturition. Present data, however, indicates that blood with high level of the inhibitor contains considerable quantities of a substance capable of causing contractions of the isolated rat's uterus and that inhibitor inactivated blood has no such quality, when the investigation is performed under the given conditions. These results strongly suggest that the increased inhibitor may have an oxytocic role on the initiation of parturition and the uterine involution after delivery.

Since Hippocrates various theories have been brought forward about the cause of labour, but none of which can be considered as satisfactory. It is now considered that labour begins as a result on the general accelerating convergence of a number of factors—structural, humoral, nervous, nutritional and circulatory (13). The appearance of the oxytocic substances in the parturient blood has been reported by many investigaters. Maruoka (10) has reported that the parturient woman blood stimulates the isolated guinea pig uterus. The period of this oxytocic action in woman blood coincides with that of NSHI rising reported by Hakanson and Click (7).

The present data are not sufficient to warrant that NSHI *per se* is an oxytocic factor. This will be elucidated in the future by the isolated NSHI. But this oxytocic substance in the serum differs from the oxytocin secreted from the posterior lobe of pituitary and from the oxytocic substances which were extracted from placental tissue by Fontes (3) and Bell *et al.* (1), because their substances are thermostable. And also it differs from histamine, because the uterus of rat is unaffected by histamine (15).

Present experiments will support the view of Eden (2) and Williams (16) that the metabolic products in the degenerated placental tissue at the end of the gestation period stimulate the uterine center.

The inhibiting action on the uterine motility was found in half cases of NSHI inactivated serum samples. Bell et al (1) has demonstrated that their blood extracts contain a factor capable of inhibiting the reaction of the uterus to oxytocin in vitro and that this inhibitory power increases on standing in ice chest. Whether the inhibiting phenomenon of NSHI inactivated serum is due to a newly produced substance by heating, or to the destraction of NSHI which has masked the antispasmodic substance in serum, is unknown.

But the slight inhibition which was found sometimes by the low NSHI level serum will suggest the possibility of the later view.

In Locke-Ringer solution the stimulating effect of NSHI on the spontaneous contractions is not so strong, though the higher NSHI level serum stimulates more than the lower serum. The ionic environment on the excitability of NSHI on the uterine smooth muscle will be studied by using purified NSHI in the future.

Summary.

1. The nonspecific hyaluronidase inhibitor (NSHI) in the pregnant rabbit's serum remained almost constant during pregnancy, but showed a marked increase between the 3rd and 4th days prepartum and reached a peak the 2nd day after the parturition, returning to the normal level within 5 to 7 days postpartum.

2. The administration of progesterone caused a decrease of NSHI in the castrated male rabbit's serum.

3. Artificial abortion of the pregnant rabbits at 23th day increased NSHI levels at the 2nd day, reached maximum values between 3rd and 4th days and returned to normal the 8th-9th days after the operation.

4. The serum of higher NSHI level stimulated the isolated rat's uterus more strongly than that of lower level in Tyrode's solution. The stimulating effect of serum on uterine motility is significantly associated with the degrees of NSHI levels.

5. NSHI inactivated serum by heating had no accelerating effect or inhibiting effect on the uterine motility.

6. The oxytocic substance in the serum which is highly associatiated with NSHI differs from oxytocin in the posterior lobe of pituitary.

7. The possible role of NSHI in the serum at parturition is discussed.

References

1) Bell, G. H. and J. M. Robson: *J. Physiol.*, **48**, 351, 1935.
2) Eden, T. W.: *J. Path. Bact.*; **4**, 265, 1897.
3) Fontes, J.: *Arch. f. Gynäk.*, **155**, 565, 1933.
4) Freeman, M. E., R. Whitney and A. Dorfman: *Proc. Soc. Exp. Biol. & Med.*, **70**, 524, 1949.
5) Glick, D. and F. Gollan: *J. Infec. Diseases*, **83**, 200, 1948.
6) Haas, E.: *J. Biol. Chem.*, **163**, 63, 1946.
7) Hakanson, E. Y. and D. Glick: *J. Clin. Jnvest.*, **28**, 713, 1949.
8) Hechter, O. and E. L. Scully: *J. Exp. Med.*, **86**, 19, 1947.
9) Ichikawa, S.: *Bull. Univ. Osaka Pref., Ser. B*, **5**, 99, 1955.
10) Maruoka, H.: *J. Jap. Gyne. Soc.*, **31**, 2433, 1936.
11) Meyer, K. and M. M. Rapport: *Advances in Enzymol.*, **13**, 199, 1952.
12) Ragan, C. and De La Mater, A.: *Proc. Soc. Exp. Biol. & Med.*, **50**, 349, 1942.
13) Reynolds, S. R. M.: *Physiology of the uterus, 2nd Ed. Hoeber*, p. 529, 1949.
14) Seifter, J., D. H. Baeder and A. J. Begany: *Proc. Soc. Exp. Biol. & Med.*, **72**, 277, 1949.
15) Sugimoto, T.: *Folia Pharm. Jap.*, **24**, 114, 1937.
16) Williams, J. W.: *Obstetrics, Lond.*, 1930.