大阪公立大学
Osaka Metropolitan University
学術情報リポジトリ

ラット血清中の非特異性ヒアルロニダーゼ抑制物質に対するステロイドホルモンの影響

メタデータ	言語: eng 出版者: 公開日: 2009-08-25 キーワード (Ja): キーワード (En): 作成者: 市川, 茂孝 メールアドレス: 所属:
URL	https://doi.org/10.24729/00009646

Bull. Univ. Osaka Pref.,
Ser. B, Vol. 5, 1955

Effects of Steroid Hormones on the Nonspecific Hyaluronidase Inhibitor in the Rat's Serum

By

Shigetaka ICHIKAWA

Department of Veterinary Science, College of Agriculture

(Received September 30, 1955)

McClean (14), Hobby *et al.* (11) and other investigators have reported the existence of the nonspecific hyaluronidase inhibitor (NSHI) in the normal blood sera of various animals. This inhibitor level in serum elevates in certain physiological and pathological situations.

Dorfman and Moses (3) and Adams *et al.* (1) reported the diminishing effect of ACTH upon the augumented hyaluronidase inhibitor levels in the sera of rheumatic fever patients. Cole and Holden (2), however, could not prevent the increase of NSHI in the dog's serum following surgical trauma. According to Good *et al.* (9) the situation in which the level of NSHI is increased was considered to represent situation of stress provocative of adrenal hyperactivity, and they obtained a concept that the adrenals play a role in the regulation of the serum inhibitor level.

Present experiments have been attempted to study the effects of some steroid hormones on NSHI concentration in the serum.

Materials and methods

EXPERIMENTAL ANIMALS.

Wistar strain rats bred in our laboratory were used throughout all experiments.

PREPARATION OF TEST SUBSTANCES.

Enzyme; hyaluronidase (H-ase) preparation as standard was obtained from the bull testicular tissue following the method of Madinaveitia and Quibell (14) and of Freeman *et al* (5). The minced bovine testis is extracted with 0.1 N acetic acid. The ammonium sulfate fraction between 0.3 and 0.7 saturation is obtained from this extract solution. This preliminary fractional precipitate is dissolved in water and dialysed against cooled distilled water. This aqueous solution is fractionated again with ammonium sulfate between 0.36 and 0.60 saturation. The precipitate is dissolved in water and dialysed as previously. This enzyme solution is added with cooled acetone at 0°C and the precipitate is dried in vacuo.

Substrate; hyaluronic acid was prepared from human umbilical cords according to the procedures described by Quinn (16) and Jeanloz & Forchielli (12). The cords stored in acetone are ground in a grinder. Then extracted with water at 0°C by agitating sometimes. The viscous supernatant fluid is separated by centrifugation. The residue is extracted once more in this fashion. The conbined fluid is adjusted to a PH 9–10 with potassium hydroxide, then 1.25 volumes of cold ethylalcohol saturated with potassium acetate are added slowly to the cooled extracts. The precipitate is washed

several times with cooled ethanol, then with cold ether and dried in vacuo. This crude preparation is dissolved in water and eliminated of residual proteins by Sévag's procedure in which the mixture of 3.3 liters of chloroform, 1.7 liters of amylalcohol, 1.0 liter of a water containing 300 g of sodium acetate and 160 g of glacial acid, is added to 5 liters of crude hyaluronic acid solution and shaked severely for about 10 minutes and then centrifuged. The supernatant aqueous phase is treated repeatedly with the same Sévag's solution until no more precipitate appear at the interface. Then the aqueous phase is decanted and two volumes of 95% ethanol is added. The precipitate is washed with alcohol for several times, then with ether and dried in vacuo.

METHOD FOR MEASUREMENT.

For the measurement of NSHI concentration in serum the viscosimetric technique described by Haas (10) and Glick & Gollan (7) was modified as follows.

Substrate solution; 2.0 mg of hyaluronic acid is dissolved in 1.0 ml of phosphate acetate buffer which consists of 1 volume of 2 M NaCl solution, 2 volumes of 0.5 M phosphate buffer (PH, 7.0) and 4 volumes of 0.02 M acetate buffer (PH, 4.7).

Standard enzyme solution; standard H-ase preparation is dissolved in 0.2 M borate buffer (pH, 6.7) and the activity was adjusted for an R_0 value to fall in the range 240–260 sec., i.e. 4.0–4.33 min. R_0 is defined as the time required to reduce the relative viscosity of the reaction mixture in which no inhibitor is present, to half of the relative viscosity of the reaction mixture without the enzyme.

Serum solution; 90×10^{-5} M $MgCl_2$ solution is used following the description of Freeman et. al (6).

PROCEDURES FOR TESTS.

The blood samples collected by cardiac puncture, are allowed to clot at room temperature for about 20 minutes, and centrifuged for 10 minutes. Sera are then removed and stored in the refrigerator at 0°C until used. 0.04 ml of serum is diluted in 1.0 ml of $MgCl_2$ solution (25-fold dilution). This serum solution is mixed with 0.5 ml of standard enzyme solution and incubated at 37.5°C for 10 minutes, so that Mg ion concentration in this reaction mixture is 60×10^{-5} millimoles per 1 ml. At the end of this period 1.0 ml of this reaction mixture is added into 2.0 ml of the substrate solution which had already been broght to 37.5°C, and the measurement of reaction time is started. 2.0 ml of this enzyme-substrate mixture is pipetted into the Ostwald viscosimeter previously set in the 37.5°C thermostat and outflow times are repeatedly measured with a stopwatch until the relative viscocity had fallen to less than one-half of that of the reaction mixture devoid of the enzyme. The reaction time required for the relative viscosity to fall to one-half of the relative viscosity of the reaction mixture without enzyme is designated R. When a R value is grater than 350 seconds the inhibitor solution was diluted sufficiently to bring the value into this range.

CALCULATION OF NSHI TITRE.

The activity of H-ase inhibitor is calculated by the formula;

$$(1/R_0-1/R)\times20\times\text{dilution rate (usualy 25 fold)},$$

where R_0 and R values are expressed by minutes (4). This value is defined as H-ase inhibitor level per ml of serum arbitrarily. The results presented here were expressed in terms of viscosity reducing units (vru) of inhibitor per ml of serum.

In all experiments the control blood samples were handled in exactly the same fashion as the experimental samples, care being taken to run both experimental and control determinations at the same time with the same preparations of enzyme and substrate. The assay of NSHI activity has finished within 4 hours after the blood has been collected.

The control values obtained in one experiment, somewhat differ from others owing to the changing of substrate.

The weights of adrenal glands, thymus, uterus or seminal vesicles are measured. Adrenal weight is expressed as AD/W ratio which means milligram weight of two glands per 100 grams body weight. The weight of the seminal vesicles are measured 24 hours after the fixation of the tissue with Bouin's solution.

Data presented in tables are expressed in mean values and unbiased estimates of standard deviations which were calculated by the formula;

$$\sqrt{\frac{1}{N-1}\Sigma(X-\overline{X})^2}.$$

Experiments and results

EXPERIMENT 1. THE CHANGES OF NSHI LEVELS BY AGE IN THE MALE RATS SERA.

This experiment is directed to the study of the NSHI levels in the sera at the various ages of the normal male rats. The results are presented in Figure 1. Until 30 days of age NSHI levels are in the range between 10 vru and 25 vru, and after this age they increase drastically and reach to the adult level. The weight of the adrenals per 100 grams of body weight (AD/W) also shows a drastic change between 30 and 35 days old. The sample correlation coefficient of the two measurements is -0.848 (degree of freedom is 28), this value means the significant negative correlation ($P<0.01$).

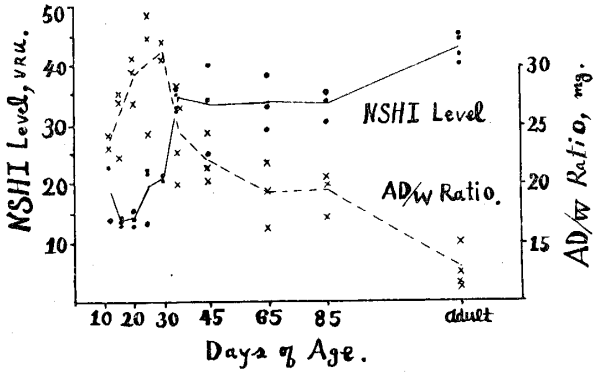

Fig. 1. The relation between NSHI level and the weight of adrenal glands per 100 g body weight at growing age of the male rats.

EXPERIMENT 2. THE INFLUENCES OF THE FORMALIN STRESS.

This experiment is designed to study the relation of NSHI level to the weight of adrenal glands under the formalin stress which is one of the most severe chemical stresses.

Eight litters of rats weighing 110 to 190 grams were divided into two groups. Two male litters and two female litters per group were selected and each litter consist of five animals. One group was used for the short period stress (24 hours) and the other for the long period stress (15 days).

Short period stress.

Four animals in each litter were subcutaneously injected in the dorsal skin with the following dosages of 1.5% formalin solution and the other one animal received no injection and served as control, so that each litter consists of one control and four different dosage administered rats. The doses and injection times per animal are;

1 injection of 1.0 ml,

2 injections of 0.5 ml with the 6 hours interval,

3 injections of 0.33 ml with the 6 hours interval,

and 4 injections of 0.25 ml with the 6 hours interval.

So that each animal was injected with 1.0 ml of formalin solution on the total volume. Six hours after the last injection they were used for the examinations.

Long period stress.

0.5 ml of 1.5% formalin solution was subcutaneously injected twice daily (at 8.00 AM and 5.00 PM) for each rat in the same litter during 2 days, 6 days, 12 days and 15 days respectively, and the other animals were not treated and served as controls. All animals were not fed during the last 24 hours except 1% saline *ad libitum.*

The initiation of injections in both short and long experiments were designed for measurments to be at the same time. Blood sugar was measured by Hagedorn Jensen method.

The various data are summarized in the Figure 2 in which four control values in the long period experiment are omitted. In the male rats the AD/W ratio decreases on the 6th hour after the formalin injection and returns to the normal value after 18 hours. In the female it tends to decrease until 12 hours after and then it increases. Blood sugar concentration increases synchroneously with AD/W ratio during 6 to 18 hours and returns back to the normal level after 24 hours. Thymus weight per 100 g body weight shows a decrease after 6 hours and returns to normal weight after 24 hours. The NSHI levels decrease after 6 hours reaching the minimum levels after 12 hours in both sexes and then they are tending to rise.

In the long term formalin administration blood sugar levels of the male rats show almost no variations, but of the female some slight increases are found. Both the AD/W ratio and NSHI levels are increasing and the thymus weight is decreasing as the injections continue.

The sample correlation coefficient between the AD/W ratio and the NSHI level calculated with 30 animals except controls, is 0.541 (d.f. 28) which indicates a significant correlation at 0.01 level. Every animal receiving continuous injec-

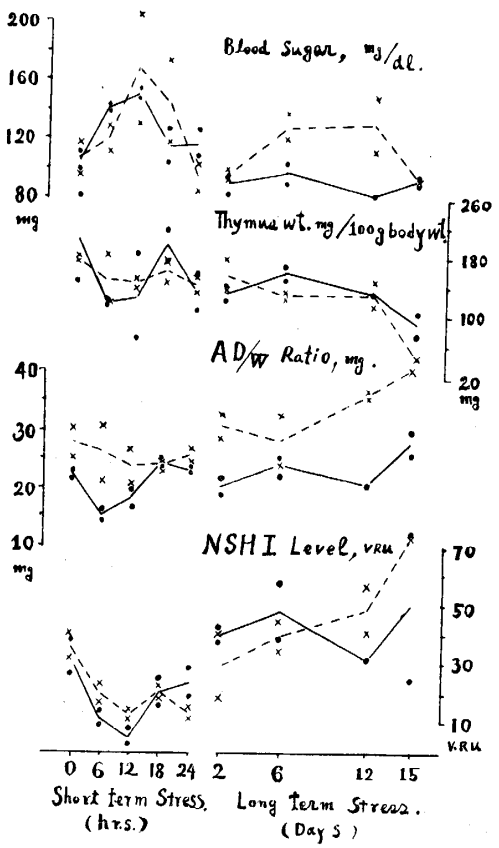

Fig. 2. Correlating changes in NSHI level, the weight of adrenals per 100 g of body weight, the weight of thymus per 100 g of body weight and blood sugar under the formalin stress.

● ; represents individual measurment of male rats and unbroken line connects their means. × ; represents individual measurment of female rats and dotted line connects their means.

tions for more than 10 days suffered from severe edema on their back.

EXPERIMENT 3. EFFECT OF ADRENALIN ON NSHI LEVELS IN SERA.

As the adrenalin secretion is expected after the formalin stress, the effect of epinephrin on the NSHI levels was studied. For this exreriment 5 adrenalectomized female rats received intramuscular injection of 0.04 mg of adrenalin chloride per 100 g body weight the 10th day after the operation. Three adrenalectomized rats and 3 sham-operated rats were used as controls. All animals were fed *ad libitum* and maintained on 1% saline after operation. After a various interval of time ranging from 3 to 30 minutes, all animals were sacrificed and blood sugar concentrations, NSHI levels, thymus weights were measured. As the data shown on Table 1 all animals epinephrine administered revealed

Table 1. Effect of adrenalin treatment on blood glucose, NSHI level, and thymus weight of adrenalectomized rats.

Treatments	No. of rat.	Times passed after inject., minutes.	Blood sugar, mg/dl.	NSHI level, vru.	Thymus wt., mg.
Adrenalectomized, adrenalin injected.	1	7	141	6.0	415
	2	15	189	20.4	431
	3	30	156	—	440
	4*	30	141	18.0	450
	5**	3	149	22.6	332
Adrenalectomized control	6	—	125	20.4	290
	7	—	100	18.0	300
	8†	—	111	39.5	65
Sham-operated control.	9	—	105	32.0	417
	10	—	108	42.7	266
	11	—	102	45.5	150

* 2 doses of 0.04 mg of adrenalin per 100 g body wt. administered i.m. with 30 minutes interval.

** 0.01 mg of adrenalin per 100 g body wt. was administered i.v.

† recognized a hypertrophied adrenal residue on the right side.

hyperglycemia. But the NSHI levels indicate no significant difference between 4 treated rats and 2 adrenalectomized untreated rats (No. 8 is omitted). The difference of NSHI levels between 2 adrenalectomized controls and 4 sham-operated controls (includes No. 8 rat) is significant.

EXPERIMENT 4. EFFECT OF DIETHYLSTILBESTROL ON NSHI LEVELS.

Gonadectomized rats.

Six male and six female rats were involved in this experiment. Two months after gonadectomy they were divided into two groups. Each animal in one group received intramuscular injection of 0.15 mg of diethylstibestrol (Takeda Pharmacological Industry) daily for 7 days. The other 6 controls simultaneously received the same volume of cotton seed oil. Three hours after the last injection all animals were sacrificed and adrenal weights, thymus weights and NSHI levels were measured.

Table 2 shows the results with the additional data of 6 untreated intact rats. Statistical examinations of the NSHI levels and the AD/W ratios in this data by analysis of variances method revealed; a) castration increased the male NSHI levels ($P<0.01$), b) spaying had no influence upon the female NSHI levels, c) diethylstilbestrol administration

Table 2. Effect of diethylstilbestrol treatment on adrenals, thymus weight and NSHI level of gonadectomized rats.

Treatments	No. of rats	Sex.	mg wt. of adrenals per 100g body wt.	Thymus wt., mg.	NSHI level, vru.
Gonad-ectomized 0.15 mg of DS, daily.	3	male	36.4± 5.13	175.0± 39.06	39.9±10.10
	3	female	48.8±11.36	163.7± 26.50	27.7± 3.37
Gonad-ectomized cotton seed oil, daily.	3	male	26.1± 6.89	330.0±130.00	48.9± 4.98
	3	female	27.4± 1.00	342.3± 45.17	35.6± 3.94
Normal control.	3	male	19.7± 3.42	130.3± 59.36	32.0± 5.66
	3	female	24.4± 5.54	252.0± 39.68	37.0±10.51

decreased the NSHI levels in male and female ($P<0.05$), d) by the gonadectomy, male adrenal glands hypertrophied and there was no significant difference of the AD/W ratios between male and female ($P>0.25$) and e) diethylstilbestrol treatment increased AD/W ratio ($P<0,01$). Thymus weights showed a significant atrophy by the stilbestrol administration ($P<0.005$).

Adrenal-gonadectomized rats.

Castrated rats weighing 150–200 g were extirpated adrenal glands in two stages, first extirpation was done one month after the castration, the 2nd operation after a period of 4 days and 4 days after the 2nd extirpation each animals received the intramuscular injection of 0.15 mg of diethylstibestrol and same volume of soy-bean oil respectively for 7 days. Three hours after the last injection all animals were examined.

Table 3. Effect of diethylstilbestrol treatment on adrenal, thymus, seminal vesicle, uterine weight and NSHI level of adrenal-gonadectomized and hypophys-gonadectomized rats.

Treatments	No. of rats	Sex.	Adrenal wt., mg.	Thymus wt., mg.	Seminal ves. or uterine wt., mg.	NSHI level, vru.
Adrenal-gonadectomized, 0.15 mg of DS/day i.m. for 7 days.	4	♂	—	343.0± 88.08	65.5±13.77	20.5±2.77
Adrenal-gonadectomized control.	3	♂	—	611.0±197.45	57.7±27.83	20.4±4.95
Adrenal-gonadectomized, 0.15 mg of DS/day i.m. for 7 days.	4	♀	—	321.0± 95.21*	269.8±42.28**	18.0±2.36
Adrenal-gonadectomized control.	3	♀	—	521.0± 98.36	70.0±20.00	21.3±2.77
Hypophys-gonadectomized, 0.15 mg of DS/day i.m. for 7 days.	4	♂	11.0±1.69	177.2± 40.12	31.5±14.17	21.4±1.51
Hypophys-gonadectomized, control.	3	♂	14.5±6.14	165.7± 73.98	20.7± 3.77	19.0±2.00
Hypophys-gonadectomized, 0.15 mg of DS/day i.m. for 7 days.	3	♀	16.2±6.22	190.7± 23.77	239.7±29.16**	20.0±3.24
Hypophys-gonadectomized, control.	4	♀	13.6±2.34	177.0± 25.75	78.5±50.39	19.2±1.72

*, ** Difference of means is statistically significant on the level $P<0.05$ and $P<0.01$ respectively.

The results are shown in Table 3. The weights of thymus glands decreased significantly in female ($P<0.05$), but NSHI levels in sera received no influence by administration of diethylstilbestrol when both adrenals and gonads are ectomized.

Hypophys-gonadectomized rats.

Castrated rats weighing 100–150 g were hypophysectomized one month after gonadectomy. Twenty days after hypophysectomy 0.15 mg of diethylstilbestrol was daily injected intramuscularly for 7 days and 3 hours after the last injection all animals were examined. The results are shown in Table 3. NSHI level received no influence as in adrenal-gonadectomized rats. Thymus weight of the hypophys-gonadectomized rats was smaller than that of adrenal-gonadectomized rats and received no influence with estrogen treatment.

EXPERIMENT 5. EFFECT OF PROGESTERONE ON NSHI LEVELS IN RATS' SERA.

Gonadectomized rats.

Thirty days after castration each doses of 2 mg or 1 mg of progesterone (Teikoku Hormone MFG., Co.) or soy-bean oil was respectively injected intramuscularly for 3 days. Three hours after the last injection all animals were examined. Body weights at sacrifice were about 150–200 g. The results are presented in Table 4.

Table 4. Effect of progesterone treatment on adrenal, thymus, seminal vesicle, uterine weight and NSHI level of gonadectomized and adrenal-gonadectomized rats.

Treatments	No. of rats	Sex.	AD/W ratio, mg.	Thymus wt., mg.	Seminal ves. or uterine wt., mg.	NSHI level, vru.
Gonadectomized rats.						
2 mg of progest.	4	♂	19.0±2.52	456.2±133.19	12.0± 2.00	34.8±6.15
1 mg of progest.	4	♂	18.1±2.33	470.0±160.79	14.5± 5.26	23.1±4.04*
Soy-bean oil.	4	♂	17.9±2.81	443.2± 94.81	35.8±29.33	36.2±9.83
2 mg of progest.	4	♀	21.0±4.15	326.0± 75.17	139.2±94.52**	25.9±5.31*
1 mg of progest.	4	♀	21.5±1.91	512.5±139.18	73.5±39.43‡	23.4±4.01*
Soy-bean oil.	4	♀	19.9±0.98	392.0± 54.76	68.3±7.06	33.9±1.08
Adrenal-gonadectomized rats.						
1 mg of progest.	4	♂	—	565.5±220.97	13.5± 4.51	17.6±4.00†
Soy-bean oil.	4	♂	—	527.5±185.03	12.5± 5.06	19.7±1.06
1 mg of progest.	5	♀	—	600.4±218.84†	64.8±11.94*	24.9±5.80‡
Soy-bean oil.	5	♀	—	624.2± 74.37	46.0±10.63	20.7±1.79

*, ** Difference of mean over control is statistically significant on the level $P<0.05$ and $P<0.01$ respectively.

†, ‡ Difference of variance over control is statistically significant on the level $P<0.05$ and $P<0.025$ respectively.

The progesterone administration induced a significant decrease of NSHI concentrations in gonadectomized rats' sera, except 2 mg administered male group. Progesterone did not affect on the adrenal weight.

Adrenal-gonadectomized rats.

Thirty days after castration adrenal glands were ectomized in 2nd stages as in

Exp. 4 and 4 days after the last operation 1 mg of progesterone was injected intramuscularly for 3 days. Adrenal-gonadectomized rats were treated with soy-bean oil as controls. The body weights at sacrifice were about 150–200 g.

The results are shown in Table 4. NSHI levels of some rats decreased and of others showed no change or increase by progesterone treatment. This indefinite tendency shows that the difference of variances is statistically significant in contrast with the almost constant levels in adrenalectomized rats in all cases.

EXPERIMENT 6. EFFECTS OF TESTOSTERONE AND METHYLANDROSTENEDIOL ON NSHI LEVELS.

Castrated rats.

One hundred and thirty days after the castration 5 male rats were used. Three castrated rats received intramuscular injections of various doses of testosterone propionate (Teikoku Hormone MFG., Co,) daily for 4 days, two castrated controls and 2 intact controls received 0.5 ml of soy-bean oil simultaneously. Three hours after the last injection animals were sacrificed and examined.

The results of each individuals are presented in Table 5. The adrenalhypertrophy

Table 5. Effect of testosterone propionate treatment on seminal vesicle, adrenals, thymus weight and NSHI level of the castrated rats.

No. of rat	Treatment	Body wt., g.	Seminal vescle wt., mg.	AD/W ratio, mg.	Thymus wt., mg.	NSHI level, vru.
Castrated rats.						
1	0.5 mg of TP daily.	194	408	13.9	150	33.3
2	0.3 mg of TP daily.	212	425	14.4	26	36.8
3	0.1 mg of TP daily.	220	329	14.8	285	38.7
4	Soy-bean oil daily.	206	38	17.9	283	51.3
5	"	212	92	16.3	280	51.3
Intact rats.						
6	Soy-bean oil daily.	215	2701	13.9	94	43.7
7	"	222	2068	14.0	147	45.3

owing to castration was prevented by the administration of 0.1 mg or more of testosterone propionate (TP) daily. Thymus also showed the same tendency. The elevated NSHI levels by castration were decreased by TP administration in proportion to the dosages.

Adrenal-gonadectomized rats.

Thirty days after castration adrenal glands of 21 male rats were extirpated in 2 stages as in Exp. 4 and 4 days after the last operation each animals received intramuscular injection of various doses of TP and methylandrostenediol (Shionogi & Co.) and soy-bean oil daily for 4 days. Three hours after the last injection animals were sacrificed and examined.

The results are presented in Table 6. TP treatment, except 1.0 mg administered group, changed NSHI levels without definite tendency (decrease or increase), showing a significant differences in the variances. 3.0 mg and 2.0 mg of methylandrostenediol (MA) administration daily for 4 days significantly decreased NSHI levels ($P<0.01$) and 1.0 mg

of MA showed the significant difference of variances in contrast with control. MA increased seminal vesicle weight showing some androgenic effect in rats.

Table 6. Effects of testosterone propionate and methylandrostenediol on adrenals, thymus, seminal vesicle weight and NSHI level of adrenal-gonadectomized and hypophys-gonadectomized male rats.

Treatments	No. of rats	Adrenals wt., mg.	Thymus wt., mg.	Seminal ves. wt., mg.	NSHI level, vru.
Adrenal-gonadectomized rats.					
1.0 mg of TP.	3	—	509.0± 60.85	178.7±44.04**	18.0±1.39
0.5 mg of TP.	3	—	430.0±251.08	157.0±17.52**	23.4±8.82‡
0.3 mg of TP.	3	—	336.0± 73.57*	184.0±33.04**	17.4±6.25†
3.0 mg of MA.	3	—	413.7± 14.69	77.0±11.91**	9.0±3.82**
2.0 mg of MA.	3	—	613.3±126.00	116.0±31.51**	11.1±2.91**
1.0 mg of MA.	2	—	572.5± 45.72	48.0±12.73	20.8±6.01†
Soy-bean oil.	4	—	585.3±142.70	30.0±12.27	19.0±1.29
Hypophys-gonadectomized rats.					
0.5 mg of TP.	4	10.0±1.41	282.8± 64.28	29.8± 3.31**	17.7±11.54‡
Soy-bean oil.	4	9.3±0.95	282.5±111.03	11.5± 2.38	20.4± 1.85

*, ** Difference of mean over control is statistically significant on the level $P<0.05$ and $P<0.01$ respectively.

†, ‡ Difference of variance over control is statistically significant on the level $P<0.025$ and $P<0.01$ respectively.

Hypophys-gonadectomized rats.

Eight rats have been hypophysectomized 30 days after castration. Twenty days after hypophysectomy 4 animals received intramuscular injection of 0.5 mg of TP daily for 4 days. The other 4 rats injected soy-bean oil as controls.

The results are shown in Table 6. In this case as in adrenal-gonadectomized rats TP administration significantly changed NSHI levels without definite tendency.

Discussion

In the normal rats at various ages a negative correlationship between NSHI levels and adrenal glands' weights per unit body weight existed, and drastic changes of NSHI level and adrenal glands' weight were found at 30–35 days of age. These results suggest that on the normal condition adrenal activity has a intimate relation with NSHI level and seems to modify the inhibitor level. On the contrary under a severe stress condition the correlationship between the AD/W ratio and NSHI level was positive. The decreased values of NSHI at 6–12 hours after formalin stress can not be ascribed to adrenalin secretion, though the increased blood sugar concentration at this period may be due to the effects of hypersecreted adrenalin and cortical hormones, because the administration of adrenalin on the adrenalectomized rats did not decrease NSHI levels. The nature of NSHI is considered as an acid polysaccharide-protein compound and this polysaccharide prosthetic group permits NSHI to act as a competitive inhibitor (8, 15, 17). So that it might be not impossible to consider that NSHI will be affected by the adrenal cortical hormones. In

fact the treatment of ACTH diminished the augumented H-ase inhibitor levels in the sera of rheumatic fever patients as previosaly mentioned. And according to Good *et al.* (9) cortisone administration increased NSHI levels in normal rats and monkeys, and they mentioned the need of the mediation of adrenal cortex for the increase of H-ase inhibitor in serum. The cause of the opposite phenomenon found in the present two results is obscure. However it may be explained by the following hypothesis. The cortical hormenes has two actions with NSHI in the serum; one is to enhance the migration of one or more components of NSHI constituents and another is to accelerate the catabolism of NSHI. And NSHI level in the serum is determined by these two actions of cortical hormones and by the tissual conditions. Under the normal condition these two actions are in the normal balance keeping the normal NSHI level. When the secretion of the cortical hormones is increased and there is no tissue destruction as in the younger ages, catobolic action is dominant and NSHI level is low. During the first 12 hours of the formalin stress (Selye's shock phase in the general adaptation syndrome) catabolic action is dominant owing to the drastic hypersecretion of cortical hormones and to the little tissue destruction. After two days of stress (Selye's stage of resistance) the migration of NSHI from the connective tissue will become dominant owing to the increased tissue degeneration, then NSHI level in the serum increases. Thus adrenal gland regulates the NSHI level homeostatically. But with this hypothesis we can not explain the increase phenomenon of NSHI in rat's serum under the cold stress where there is no tissue destruction, reported by Good *et al.*

When adrenals are extirpated the migration of NSHI from the connective tissue is reduced to the minimum level which is almost constant. Then the adrenal gland is essential for the increase in the H-ase inhibitor in blood as Good *et al.* have related.

The administration of large dose of diethylstibestrol, 0.15 mg daily for 7 days, decreased NSHI level in gonadectomized male and female rats' sera accompanying the hypertrophy of adrenal glands and the involution of thymus gland. Diethylstilbestrol, however, could not change NSHI levels both in adrenal and hypophysectomized rats. Then it may be considered that the NSHI diminishing effect of this hormone in rat's serum is mediated by the hypophys-adrenal cortex system.

Testes extirpation increased NSHI level and induced the hypertrophy of adrenals in male rat. Testosterone propionate treatment resulted the decrease of the inhibitor level and the involutions of hypertrophied adrenal glands and thymus in gonadectomized rats. And TP changed NSHI levels in the sera without definite tendency, some increased and others decreased, both in hypophysectomized and in adrenalectomized rats. Methylandrostenediol decreased NSHI levels in adrenalectomized rats. Progesterone also decreased NSHI levels in gonadectomized rats' sera and changed NSHI levels in adrenalectomized rats as in the same fashion with TP, but had no influences on adrenal and thymus weights. These results indicate that TP, MA and progesterone affect NSHI levels without midiation of adrenal. The reason why these hormones decrease NSHI levels in some sera and increase in other sera, is not yet known. Whether these effects of the steroid hormones on NSHI

levels in sera are due to the protein anabolic effect of testosterone and methylandrostenediol or due to the corticomimetic effect of progesterone, or not, are also yet unknown.

Summary

1. The viscosimetric technique for the measurement of hyaluronidase inhibitor in the serum was decribed and effects of several steroid hormones on the nonspecific hyaluronidase inhibitor (NSHI) levels in rats' sera have been studied.

2. NSHI levels and the weights of adrenals per 100 g of body weight in the normal male rats showed a drastic change between 30 and 35 days of age and these two measurements showed a significant negative correlationship.

3. Under the formalin stress, NSHI levels, adrenal weights and thymus weights decreased and blood sugar increased synchronecusly during the first 12 hours and these values returned to the normal levels until 24 hours after the initiation of the stress. And NSHI levels and adrenals weight continued to increasing thereafter. NSHI concentrations and adrenal weights per 100 g of body weight showed a significant positive correlationship.

4. Diethylstilbestrol injection decreased NSHI levels in sera of gonadectomized rats. But it has no effect on NSHI of the adrenal-gonadectomized and hypophys-gonadectomized rats.

5. Progesterone admistration decreased NSHI levels in sera of gonadectomized rats. and changed the inhibitor levels of adrenal-gonadectomized rats without definite tendency.

6. Testosterone propionate treatment induced the decrease of NSHI levels in the gonadectomized rats' sera, and it changed NSHI levels of adrenal-gonadectomized rats and hypophys-gonadectomized rats showing a significant differences of variances in contrast with controls.

References

1) ADAMS, F. H., V. C. KELLEY, P. F. DWAN and D. GLICK: *Pediatrics*, **7**: 472, 1951.
2) COLE, J. W. and W. D. HOLDEN: *Proc. Soc. Exp. Biol. Med.*, **77**: 363, 1951.
3) DORFMAN, A. and F. E. MOSES: *ibid.*, **73**: 667, 1950.
4) DORFMAN, A., M. L. OTT and R. WHITNEY: *J. Biol. Chem.*, **174**: 621, 1948.
5) FREEMAN, M. E., P. ANDERSON, M. OBERG and A. DORFMAN: *ibid.* **180**: 655, 1949.
6) FREEMAN, M. E., R. WHITNEY and A. DORFMAN: *Proc. Soc. Exp. Biol. Med.*, **70**: 524, 1949.
7) GLICK, D. and F. GOLLAN: *J. Inf. Dis.*, **83**: 200, 1948.
8) GLICK, D. and B. SYLVÉN: *Science*, **113**: 388, 1951.
9) GOOD, T. A., R. A. GOOD, V. C. KELLEY and D. GLICK: *Am. J. Physiol.*, **166**: 555, 1951.
10) HAAS, E.: *J. Biol. Chem.*, **163**: 63, 1946.
11) HOBBY, G. L., M. H. DAWSON, K. MEYER and E. CHAFFEE: *J. Exp. Med.*, **73**: 109, 1941
12) JEANLOZ, R. W. and E. FORCHIELLI: *J. Biol. Chem.*, **186**: 495, 1950.
13) MADINAVEITIA, J. and T. H. H. QUIBELL: *Biochem. J.*, **34**: 625, 1940.
14) MCCLEAN, D.: *J. Path. Bact.*, **53**: 284, 1942.
15) MOORE, D. H. and J. N. HARRIS: *J. Biol. Chem.*, **179**: 377, 1949.
16) QUINN, R. W.: *J. Clin. Invest.*, **27**: 463, 1948.
17) WATTENBERG, L. W. and D. GLICK: *Arch. Biochem. Biophys.*, **35**: 290, 1952.