Effect of Estrogens on Blood Clotting and Plasmin Systems*

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In order to clarify the controversial findings concerned with the effect of estrogens on coagulation and plasmin systems, various preparations of estrogen having different chemical structures, i.e., desoxyestrone, estradiol and Premarin R, were administered to human subjects and the factors of blood coagulation and the plasmin systems were determined.

There was no significant change in the factors of the coagulation system excepting a slight decrease in the recalcification time with the administration of estriol or Premarin R, slight elevation of Factor VII with Premarin R, and a slight prolongation of the thrombin time with estradiol, estriol and Premarin R. On the other hand, a marked change was noted in the plasmin system, namely, a decreases in the total plasmin and antiplasmin, and a marked elevation of the spontaneous caseinolytic activity of the euglobulin fraction.

A hypercoagulemic state is observed during pregnancy characterized by a decrease in the clotting time of whole blood, a decrease in r (reaction time) and K (coagulation time) and an increase of ma (maximal amplitude) in the thrombelastogram as well as increases in fibrinogen, prothrombin, Factor V and Factor VII.¹⁻⁵ At the same time, increases in the plasmin system including increases in the total plasmin (caseinolytic activity of the SK-euglobulin), and spontaneous proteolytic activities of the euglobulin fraction are also found in pregnant women,⁶⁻⁸ as if they play a role in buffering the hypercoagulemic state. These changes seem to be under neurohormonal control especially by the sex hormones. It was reported that Premarin R, a mixture of sodium salts of estrone sulfate and equilin sulfate from the urine of pregnant mares, can produce increases of fibrinogen, Factor V and Factor VII.⁹ It was also noted that estrogens have a depressing effect on the blood cholesterol level.^{10,11} If it is true, as is generally accepted, that hyperlipemia can produce a hypercoagulemic state, the above actions of

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estrogen on the clotting system might appear to be conflicting findings.

In this paper, the effect of estrogens having different chemical structures on the clotting and plasmin systems are discussed.

MATERIALS AND METHODS

1. *Materials*

Estrogens were administered to human females who, in almost all of cases lacked evidence of clinical ovarian function, and then the activities of the clotting and plasmin systems were determined successively and compared with baseline data determined prior to the administration. The dosage and method of the administration of estrogens are summarized in Table I.

TABLE I. Chemical Structure and Dosage of Estrogens and Method of the Administration

Preparation	Dosage and method of administration
Desoxyestrone (DOE)	3 mg/day, parenterally, for 6 days
Estradiol propionate depot (ED)	10 mg as one injection, intramuscularly
Estriol-3-benzoate, 16, 17 diacetate depot (ET)	10 mg as one injection, intramuscularly
Premarin R	20 mg as one injection, intravenously

2. *Methods of analyses*

- (1) Recalcification time (Stefanini)
- (2) Prothrombin time (Quick's one stage method)
- (3) Thrombin time (Hougie¹²)
- (4) Heparin tolerance test (Kikuchi¹³)
- (5) Factor V¹⁴

a) Factor V-deficient plasma was prepared by incubating at 37°C for 24 hours plasma which was obtained by centrifuging a mixture of 1 vol. of 1.5% Na₂-EDTA (sodium ethylenediamine tetraacetate) solution and 9 vols. of venous blood.

b) Adsorbed plasma: One ml of citrated plasma (supernate of centrifugate of mixture of 1 vol. of 3.8% sodium citrate and 9 vols. of venous blood) was mixed with barium sulfate while stirring for 5-6 minutes, then the supernate (adsorbed plasma) was obtained by centrifugation.

Factor V was determined on a mixture of equal vol. of Factor V-deficient plasma and adsorbed plasma by using a technique of Quick's one stage prothrombin time.

- (6) Factor VII complex¹⁵

- a) BaSO_4 -adsorbed plasma (see above)
- b) Factor VII-deficient plasma was obtained by stirring a mixture of each 0.1 ml 100 mg of Bentonite and 1 ml of citrate plasma and followed by centrifugation.

Factor VII-complex was determined on a mixture of each 0.1 ml of Factor VII-deficient plasma, BaSO_4 -adsorbed plasma (1:8 diluted), test citrated plasma (1:10 diluted) and tissue thromboplastin (Mochida Co-Lyoplastin) by adding 0.2 ml of M/40 calcium chloride at 37°C.

(7) Fibrinogen¹⁶: A modification of Ratnoff and Menzie method¹⁷

(8) Total plasmin: This was done by incubating SK-activated euglobulin with casein.¹⁸

(9) Antiplasmin: As immediate inhibitor of plasmin presented by Norman^{19,20} is closely related to slow antiplasmin, the former alone was determined by subtracting the caseinolytic activity of SM-plasma from that of SK-euglobulin.²¹

(10) Spontaneous proteolytic activities of the euglobulin fraction: These were measured by incubating a mixture of the euglobulin fraction without any addition of artificial activating procedure and casein or fibrin substrate.

(11) Antitrypsin: A modified method of Kunitz¹⁸

RESULTS

The recalcification time and the prothrombin time remained within the normal range, with the exception of a slight decrease in the recalcification time in ET and the Premarin R groups (Figs. 1 and 2).

The thrombin time was slightly increased in major parts of ED, ET and

Fig. 1. Changes in recalcification time after the administration of estrogens.

Fig. 2. Changes in prothrombin time after the administration of estrogens.

Fig. 3. Changes in thrombin time after the administration of estrogens.

Premarin groups, but rather shortened in DOE group, and finally returned to the same level as prior to administration (Fig. 3).

An irregular change, but within the normal range, was observed in the heparin tolerance test and the fibrinogen concentration in every case (Figs. 4 and 5).

The activities of Factors V and VII after the administration of Premarin R showed essentially no change in the former and slight elevation in the latter

Fig. 4. Changes in heparin tolerance test after the administration of estrogens.

Fig. 5. Changes in fibrinogen level after the administration of estrogens.

(Table II).

The total plasmin and the immediate inhibitor were simultaneously decreased after the administration of estrogens, but were somewhat irregular in the DOE and ED groups (Figs. 6 and 7).

Spontaneous fibrinolytic activity of the euglobulin fraction showed no definite change in DOE, ET and Premarin R groups, but a marked fall of the activity

TABLE II. Influence of Premarin R Injection on Factor V and Factor VII

Factors examined	Case	Time examined			
		Before administration	Days after injection		
			2	5	10
Factor V	A	15.0 (sec)	17.2 (sec)	14.4 (sec)	17.0 (sec)
	B	21.7	19.8	22.5	17.0
	C	18.2	19.2	17.6	16.9
Factor VII	D	23.0	20.2	16.1	16.4
	E	20.4	20.4	18.3	18.6
	F	20.0	20.0	18.6	18.6
	G	18.1	17.5	16.8	15.5
	H	17.2		15.5	16.8

Fig. 6. Changes in total plasmin after the administration of estrogens.

was noted in 4 out of 6 cases of ED group (Fig. 8).

A striking elevation of the spontaneous caseinolytic activity of the euglobulin fraction was observed in each group (Fig. 9).

No significant change in antitrypsin was observed in DOE, ED and Premarin groups, but a sharp rise was noted in ED group alone (Fig. 10).

The results obtained can be summarized as is presented in Table III.

DISCUSSION

The actions of estrogens on extragenital systems have been becoming of increasing interest. For example, the estrogen preparation for intravenous in-

Fig. 7. Changes in immediate inhibitor after the administration of estrogens.

Fig. 8. Changes in spontaneous fibrinolytic activity of the euglobulin fraction after the administration of estrogens.

jection, Premarin R, has been used widely as a hemostatic for endometrial, gastrointestinal and postoperative bleeding and epistaxis. Johnson⁹ observed maximal increases of Ac-globulin and prothrombin 15 minutes after the intravenous

Fig. 9. Changes in spontaneous caseinolytic activity of the euglobulin fraction after the administration of estrogens.

Fig. 10. Changes in antitrypsin activity in plasma after the administration of estrogens.

injection of Premarin R in dogs. Kawanami²² also reported an increase of prothrombin activity after giving estradiol benzoate to castrated human females. On the other hand, McGovern *et al.*²³ reported that the changes in prothrombin,

TABLE III. Summary of the Changes of Clotting and Plasmin Systems after the Administration of Estrogens

Estrogens administered	DOE	ED	ET	Premarin R
Tests				
Recalc. time	Irregular	Irregular	Decreased	Decreased
Prothrombin time	Irregular	Irregular	Prolonged	Prolonged
Thrombin time	Decreased	Prolonged	Prolonged	Prolonged
Heparin tol. test	Irregular	Irregular	Irregular	Irregular
Fibrinogen	Irregular	Irregular	Irregular	Irregular
Factor V	No change	No change	No change	No change
Factor VII	No change	No change	No change	Slightly elev.
Total plasmin	Irregular	Slight. decr.	Decreased	Decreased
Antiplasmin	Irregular	Sl. decr.	Decreased	Decreased
Fibrinolytic act. of the eug. fr.	Irregular	Markedly depressed in 3 of 5	Sl. decr.	No change
Caseinolytic act. of the eug. fr.	Elevated	Elevated	Elevated	Elevated
Antitrypsin	Irregular	No change	Markedly elevated	No change

Factor V and Factor VII were not significant following administration of Premarin R as compared with a control group. It was also noted by Firat and Stutzman²⁴ that an increase in the platelet count was not observed in thrombocytopenic patients following the administration of equine estrogens. The increases in fibrinogen, plasminogen and antiplasmin after Premarin R injection were noted by Phillips *et al.*,²⁵ but the changes seemed to be insignificant.

The clotting time as determined by various coagulation tests does not decrease concomitantly with the increases in the clotting factors, especially when the concentration of these factors are increased above the normal level. An accurate concept of the hypercoagulemic state or the increase in the individual factors is, therefore, difficult by the usual methods, with the exception of such chemical assays as in the fibrinogen determination or prothrombin assay by the TAME-lytic method,

Though, in the authors' experiment, a decrease in the recalcification time was observed in both groups of ET and Premarin R, this does not indicate an actual elevation in hemostatic activity. For example, the authors often noted a decrease in the recalcification time or the clotting time of whole blood in patients with hypofibrinogenemia in which lowered fibrinogen and other clotting factors, and a marked hemorrhagic diathesis were observed, as well as in the umbilical cord blood in which factors of the clotting systems were decreased. For these reasons the hemostatic action of estrogen may not be due to its effect on the coagulation system. Estrogens may act as repairing agents on injured tissue, especially on the endometrium. In addition, it has been known that estrogens, e.g., Premarin R, have an ability to produce an increases of acid mucopolysaccharides in the arterioles and capillaries, and their surrounding tissue.²⁶ These are

the main roles of estrogens in the hemostatic mechanism.

Assay of the plasmin system can be determined *in vitro*, in the manner of a chemical reaction with considerable accuracy in contrast to that of the clotting system. The mechanism of decrease of total plasmin by the administration of estrogens can be explained in two ways; (1) inhibition of plasminogen production, and (2) consumption of plasminogen through activation. Since the elevation of spontaneous caseinolytic activity of the euglobulin fraction was observed, the consumption of plasminogen through its activation might conceivably occur. However, the enhancement of spontaneous fibrinolytic activity of the euglobulin was not found simultaneously with that of caseinolytic activity. This finding may support the author's opinion that there might be multiple enzymes in the euglobulin fraction, such as caseinolytic-type or fibrinolytic-type plasmin.²⁷ The physiological function of the spontaneous caseinolytic activity of the euglobulin still remains completely unknown. But possibly, the improvement of climacteric complaints following the administration of estrogens, especially in patients with vasomotor disturbances, may relate the caseinolytic activity to the production of biologically active peptides such as plasma kinins.

In general, an increase of total plasmin is observed when the blood is in a hypercoagulable state, for example during pregnancy. The plasminogen seems to act, therefore, as a reserve power in dissolving the fibrin clot in the vascular wall when it happens to occur. A decrease of total plasmin can be found in the reversed situation.

From the facts that (1) estrogens caused no significant change in the clotting factors, (2) estrogens caused a decrease of total plasmin and an elevation of marked spontaneous caseinolytic activity in the euglobulin fraction, and (3) estrogens are antilipemic (this is not from the authors' experiment), it is suggested that estrogens may not exert an accelerating but rather an inhibiting influence upon the coagulation process.

References

- 1) Maki, M. *Annual Report of Tohoku obstet. & gynec. Soci.* (Jap.), 1961, **9**, 50.
- 2) Kikuchi, E., Sato, T. & Maki, M. *J. Jap. Obstet. Gynec.* (Jap.), 1961, **13**, 1113.
- 3) Kikuchi, I. *Annual Report of Tohoku obstet. & gynec. Soci.* (Jap.), 1963, **11**, 51.
- 4) Maki, M. *J. Jap. Obstet. Gynec.* (Jap.), 1964, **16**, 13.
- 5) Beller, F.K. *Die Gerinnungsverhältnisse bei der Schwangeren und beim Neugeborenen*, Johann Ambrosius Barth, Leipzig, 1957.
- 6) Elsner, P. *Fortschr. Geburtsh. Gynäk.*, 1957, **6**, 1.
- 7) Maki, M. & Nagasawa, K. *Tohoku J. exp. Med.*, 1963, **80**, 315.
- 8) Maki, M., Nagasawa, K., Nagayama, M. & Sasaki, K. *ibid.*, in press.
- 9) Johnson, J.F. *Proc. Soc. exp. Biol.*, 1957, **94**, 92.
- 10) Oliver, M.F. & Boyd, G.S. *Lancet*, 1956, **2**, 1273.
- 11) Ando, H., Taruya, M., Nakanowatari, K. & Suto, T. *Clin. Endocr.* (Jap.), 1963, **11**, 755.
- 12) Hougie, C. *Acta haemat.*, 1960, **24**, 135.

- 13) Kikuchi, I., unpublished
 - 14) Hagihara, Y. & Ono, G. *Jap. J. clin. Med.* (Jap.), 1963, **21**, 1687.
 - 15) Tagawa, T. *ibid.*, 1963, **21**, 1691.
 - 16) Maki, M., Takano, A. & Sasaki, K. *Tohoku J. exp. Med.*, in press.
 - 17) Ratnoff, O.D. & Menzie, C. *J. Lab. clin. Med.*, 1951, **37**, 316.
 - 18) Maki, M. *Tohoku J. exp. Med.*, 1963, **81**, 179.
 - 19) Norman, P.S. *J. exp. Med.*, 1958, **108**, 53.
 - 20) Norman, P.S. *ibid.*, 1958, **108**, 639.
 - 21) Maki, M., Saito, H. & Yoneya, T. *Tohoku J. exp. Med.*, 1964, **83**, 168.
 - 22) Kawanami, A. *J. Jap. Obstet. Gynec.*, 1957, **4**, 250.
 - 23) McGovern, J.J., Bunker, J.P., Goldsteen, R. & Estes, J.W. *J.A.M.A.*, 1961, **175**, 1011.
 - 24) Firat, D. & Stutzman, L. *Proc. Soc. exp. Biol.*, 1960, **103**, 474.
 - 25) Phillips, L.L., Turksoy, R.N. & Southam, A.L. *Amer. J. Obstet. Gynec.*, 1961, **82**, 1216.
 - 26) Schiff, M. & Burn, H.F. *Arch. Otolaryng.*, 1961, **73**, 43.
 - 27) Maki, M. & Nagasawa, K. *Tohoku J. exp. Med.*, 1963, **80**, 315.
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