Med. J. Cairo Univ., Vol. 62, No. 2, June (Suppl.) : 31-42, 1994.

A Study on the Role of the Male Hormone, Mesterolone, on Hemostatic Functions in the Rat

NAGAT Y. MINA, M.D.; MONA A. NESSIM, M.D.;
MAHA M. GAMAL EL-DIN, M.D. and FATMA H. SHABAKA, M.D.

The Physiology and Dermatology & Venereology Departments, Faculties of Medicine, Cairo and Al-Azhar Universities.

Abstract

The effects of male hormones on hemostasis were studied. Mesterolone was injected daily (2 mg/kg) for 4 weeks. In another group castration was done and blood was taken 6 weeks after the operation. The results were compared to normal adult male rats. The parameters used were % platelet aggregability induced by collagen, prothrombin time (PT), activated partial thromboplastin time (APTT) in seconds, fibrinogen level (FIB) in mg/100 ml and antithrombin III% (ATIII%). The results obtained showed that mesterolone stimulated platelet aggregability while castration inhibited it. Male hormones stimulate platelet aggregability most probably through increased generation of thromboxane A_2. PT, APTT and FIB showed no significant change, while ATIII% increased significantly under the influence of mesterolone. These negative results may be due to the absence of a stimulatory effect of male hormones on coagulation factors or an increase in the synthesis of some coagulation proteins occurred but was masked by the increase in ATIII%. Prolonged PT, APTT and decreased FIB level in castrated rats could be due to decrease in protein synthesis due to lack of male hormones in castrated rats.

Introduction

GONADAL steroids have been suggested to play a role in regulating platelet function in the rat and guinea pig.[**1**]. Changes in platelet aggregability in response to estrogens and progesterone have been reported[**2**]. The effect of androgens on this system, however, has not been clarified. Several reports have substantiated the hypothesis that alterations of sex hormones, especially low testosterone in men, represents a risk factor for coronary artery disease[**3,4**].

Normal male rats have been reported to yield platelets that are 10 times more responsive to aggregating agents than those removed from females of the same age[**1**]. Still, other studies indicate that male rats with experimentally induced occlusive arterial thrombosis show a significant increase in the size of the thrombus compared with female rats[**5**]. Clinical

and laboratory studies indicate that sex steroids play important roles in the metabolism of coagulation proteins[6].

The aim of the present work was to study the role of the male hormone, mesterolone, on hemostasis. The following parameters were measured 1) platelet aggregability induced by collagen. 2) Tests for blood coagulation, prothrombin time (PT), activated partial thromboplastin time (APTT) and fibrinogen (FIB) level in the blood. 3) Tests for anticoagulant activity, antithrombin III% (ATIII%) was chosen since it has a powerful and immediate antithrombin action as well as an inhibitory effect on factors IX, X, XI and XII.

Material and Methods

Adult male albino rats weighing 150-200 gram were utilized in the present work. The animals were allowed free access to food and drinking water.

Three groups were included, each containing 6 rats. (1) Control group. (2) Testosterone treated group : Mesterolone in a dose of 2 mg/kg was injected intraperitoneally every day for 4 weeks[7]. (3) Castrated group : Blood was collected 6 weeks after castration was done[8].

In all groups of experiments blood was collected retro-orbitally on trisodium citrate in a ratio of 1 : 9 (volume of citrate/volume of blood).

Determination of Platelet Aggregability :

Collagen-induced platelet aggregability was assayed according to the method of Born[9] using platelet aggregometer Coultronics (540 dual channel aggregometer and 540 dual channel recorder). The whole impedance method was the technique used during the study. Blood samples were incubated at 37°C for 10 minutes, recalcified by the addition of calcium chloride ($CaCl_2$) to give a final concentration of 1-2 mM/ml[10], then used immediately for the assay. Collagen obtained from Diamed was used as an agonist for platelet aggregation in a dose of 100 l/ml blood. The results were expressed on a chart recorder as an aggregation curve and were calculated as percentage of maximum aggregation curve which is the distance from the addition of reagent to the plateau[11].

Assessment of Blood Coagulation (PT, APTT and FIB) :

ACL-200 automated coagulation system, Instrumentation Laboratory, was used to determine these parameters, using plasma obtained by centrifugation of the collected blood samples. The ACL is a nephelometric centrifugal analyzer which measures the intensity of light scattered by a plasma (sample) before, during and after clot formation.

— *IL Test™ PT-FIB* (97567-10) :

PT assay is sensitive to deficiencies in the activities of factor II, V, VII and X. The IL Test™ PT-Fibrinogen is a lyophilized extract from rabbit brain with the addition of optimal concenration of calcium ions

— *IL TestTM APTT* (84687-10) :

APTT assay is sensitive to deficiencies in the activities of factor II, V, VII, IX, X, XI and XII. The intrinsic coagulation system is activated by micronized silica plus bovine brain cephalin, which is the substitute of platelet Factor 3. Clotting starts by the addition of calcium chloride.

Assessment of Anticoagulant Activity (ATIII%) :

ATIII (heparin cofactor) has a powerful and immediate antithrombin action in presence of heparin.

— *IL TestTM Antithrombin III* (97574-15) :

The assay is carried out in two steps : (1) incubation of the sample with an excess of thrombin in the presence of heparin, (2) detection of the residual thrombin activity on a synthetic chromogen substrate.

Statistical analysis of the results was carried out by Student's t-test.

Results

1. *Control group :*

The results showed a mean % platelet aggregation of 69.6 ± 1.33 (Table 1 & Fig. 1). The mean values of PT and APTT in seconds were 11.98 ± 0.39 and 15.6 ± 0.63 respectively. The mean value of fibrinogen level in plasma (mg/100 ml) was 296 1 ± 6.1, while mean value of ATIII % was 84.1 ± 1.77 (Table 1).

2. *Mesterolone treated group :*

The mean % of platelet aggregation was 80.5 ± 2.17, the mean values for PT and APTT in seconds were 12.3 ± 0.29 and 15.7 ± 0.8 respectively. FIB mean value was 295.3 ± 6.89 mg/100 ml and ATIII% was 160.3 ± 1.32 (Table 2). In comparison with the control group as seen in table (4), mesterolone injection resulted in a significant increase in % platelet aggregation ($P < 0.0025$) and ATIII% ($P < 0.0005$) whereas, the other parameters showed no significant changes compared to control group.

3. *Castrated group :*

The mean value % platelet aggregation was 20.8 ± 1.9, the mean values for PT and APTT were 22.5 ± 1.13 and 28.28 ± 1.35 respectively. FIB level was 183.2 ± 3.87 mg/100 ml and ATIII% was 80.8 ± 1.85 (Table 3) It can be observed that castration resulted in a significant decrease in % platelet aggregability ($P < 0.0005$), prolongation of PT and APTT ($P < 0.0005$) compared to control. ATIII% however, showed no significant change (Table 4).

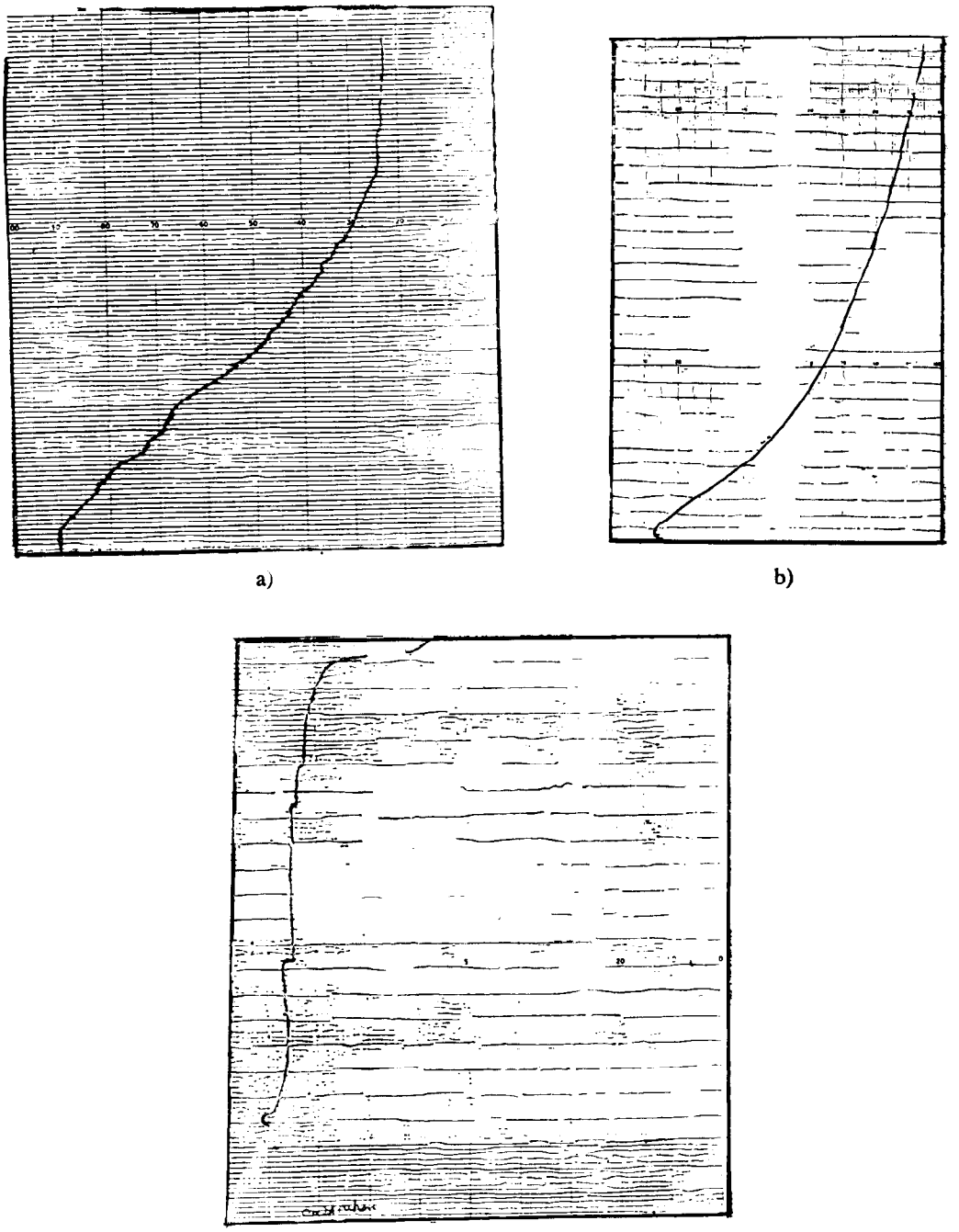

Fig. 1. Collagen induced platelet aggregability in a) control b) Mesterolone treated (4 weeks) and c) castrated group (6 weeks).

Table (1) : % Platelet Aggregation,PT in Seconds, FIB Level (mg/100 ml), APTT in Seconds and ATIII% in Control Adult Male Rats.

No. of Experiment	% Platelet Aggregation	PT seconds	FIB mg/100 ml	APTT seconds	ATIII%
1	68	11.3	315	15.8	80
2	70	11.2	312	13.1	79
3	75	10.9	288	17.3	83
4	65	12.9	290	17 1	85
5	70	13.0	276	14 9	90
6	70	12.6	296	15.9	88
Mean	69 6	11.98	296 1	15.6	84 1
S.E.	1·33	0 39	6 1	0 63	1 77

Table (2) : The Effect of Mesterolone Treatment (2 mg/kg) Daily for 4 weeks on % Platelet Aggregation, PT, FIB Level. APTT and ATIII% in Adult Male Rats.

No of Experiment	% Platelet Aggregation	PT seconds	FIB mg 100 ml	APTT seconds	ATIII%
1	76	11.1	303	14 0	158 8
2	78	12.5	265	19 4	160 2
3	89	13 0	311	14 3	155 7
4	85	12 9	296	16 2	163.4
5	76	11 9	308	15.6	164.5
6	79	12.4	289	14 9	159.2
Mean	80 5	12.3	295 3	15.7	160.3
S E	2 17	0 29	6 89	0 8	1.32

Table (3) : The Effect of Castration on % Platelet Aggregation, PT, FIB Level, APTT and ATIII% in Adult Male Rats.

No. of Experiment	% Platelet Aggregation	PT seconds	FIB mg/ 00 ml	APTT seconds	ATIII%
1	19	23 0	196	22.3	82
2	15	24 1	186	31 7	86
3	20	20 5	191	27 6	83
4	25	23 1	189	29.5	79
5	22	22 9	154	28 1	78
6	24	21 6	183	30 5	77
Mean	20 8	22 5	183.2	28.28	80.8
S E	1.9	1 13	3.87	1.35	1.85

Table (4) : Cumulative Table : Mean ± S.E. Values for % Platelet Aggregation, PT, FIB Level, APTT and ATIII% in Control. Mesterolone Treated and Castrated Adult Male Rats.

Group of Exp. / Parameter measured	% Platelet Aggregation	PT seconds	FIB mg/100 ml	APTT seconds	ATIII%
Control	69 6±1 33	11.98±0.39	296.1±6.1	15 6±0·63	84 1±1 77
Mesterolone treated group	80 5±2.17*	12.3±0.29	295.3±6.89	15.7±0 8	160.3±1.32*
Castrated group	20 8±1 9*	22.5±1 13*	183.2±3 87*	28 28±1.35*	80.8±1 85

* significant change compared to control values

Discussion

Sex steroids are known to have significant effects on platelet responses to aggregating agents as well as on the metabolism of coagulation proteins.

The results of the present work demonstrated an increase in collagen induced platelet aggregability in response to mesterolone and a decrease by castration. The present results agree with previous in vivo and in vitro studies. Thus, Johnson and coworkers[1] reported that castration reduces, whereas testosterone treatment enhances platelet aggregability and restores the reduced response in castrated male rats. In the same study, testosterone added in vitro to rat or human platelet rich plasma (PRP) was found to enhance platelet aggregability to ADP, adrenaline, collagen and arachidonic acid. Moreover, testosterone potentiates ionophore A 23187 induced aggregation in washed platelets and in PRP, an effect which is dose and time dependent[12].

These data, however, do not illuminate the specific mechanisms underlying platelet sensitivity to sex steroids. Several mechanisms have been proposed. Sex steroids are known to be absorbed at platelet membranes modifying their surface properties and inducing potential and permeability changes[13]. Previous studies suggested that sex steroids interact with various factors such as fibrinogen and plasminogen or fibrinolytic inhibitors[14]. Such reactions may be involved in that changes reported with estrogens and progestens with respect to alterations in platelet adhesiveness[15] and aggregation[16].

The involvement of prostaglandin products in mediating the stimulatory effect of testosterone on platelet aggregation was previously suggested[17]. Arachidonic acid is a known precursor of prostaglandin endoperoxides PGG_2 and PGH_2, as well as thromboxane A_2 (TxA_2) which is a powerful aggregating agent[18]. Testosterone stimulates arachidonate release and TxA_2 formation in human washed platelets[12]. This stimulation although insufficient by itself to cause platelet aggregation, does potentiate aggregation by subaggregatory doses of ionophore A 23187 and ADP. Testosterone stimulates arachidonate deacylation from platelet phospholipids but does not affect the metabolic conversion of released arachidonate to specific oxygenated products. Whether testost terone exerts only a specific stimulation of lipolytic processes or may also act directly via a non-specific detergent effect on platelet membranes is not yet known[12]. But regardless of the exact mechanism, increased generation of TxA_2 in platelets appears to be the chief metabolic event caused by testosterone since pretreatment with aspirin, a prostaglandin synthetase inhibitor, abolishes both increased TxA_2 formation and the testosterone potentiating effect on ionophore or ADP induced aggregation[12]. Moreover, Spranger et al. [19] postulated that the inhibitory effect of aspirin on platelets aggregation is greater in men than women and is reduced in orchiectomized male patients and restored by the addition of testosterone to blood samples.

PT, APTT and FIB showed no significant change by mesterolone treatment

while ATIII activity was significantly increased. Those results agree with previous studies, thus, studying the effect of testosterone on the synthesis and plasma levels of selected coagulation proteins, Owens and his coworkers[20] used isolated rat liver perfused in vitro for 10 hours. They reported that neither the biosynthesis nor plasma concentrations of factor II, VII, ATIII, plasminogen or fibrinogen were significantly affected by different doses of testosterone used. The only positive result they obtained was an increase in biosynthesis of fibronectin by increasing doses of testosterone. In a more recent study no direct relation was found between the assay of factor VIII and vWF-Ag and the levels of oestradiol, testosterone or progesterone [21].

Increased ATIII activity is in accord with Klöcking et al.[7], but is contrary to other studies which reported no change in patients with metastatic prostate cancer[22].

Klöking et al.[7] studied the ability of anabolic steroids to enhance fibrinolysis. After daily administration of 2 mg/kg for 12 weeks, the anabolic steroids were found to enhance spontaneous fibrinolytic activity of the blood, the fibrinolytic capacity and tissue plasminogen activator activity in the kidneys of rats and rabbits. Concomitantly the ATIII activity was significantly enhanced. Those changes measured in the fibrinolytic system were explained by enhancement of the biosynthesis of plasminogen activator which is assumed to be an expression of protein synthesis stimulated by steroids. However, not all factors of the fibrinolytic system respond to an enhancement of protein synthesis in a similar manner.

Al Mondhiry[22] studied the hemostatic effects of androgen treatment in 10 patients with metastatic prostate cancer. It was found that base line levels of plasma beta thromboglobulin (BTG), platelet factor 4 (PF4), fibrinogen, fibrin (ogen) split products (FSP), factor VIII coagulant activity (VIIIc), von Willebrand factor antigen (VWF Ag) and fibrinopeptide A (FPA) are significantly elevated compared to control healthy individuals. Androgen stimulation resulted in elevation of BTG, FPA and FSP levels in many patients, the changes for the entire group were not statistically significant. Other parameters remained unchanged or were slightly elevated. It was concluded that androgen stimulation of this hormonally dependent tumour may cause further activation of platelets, coagulation and fibrinolysis.

Castration of male rats resulted in prolongation of PT and APTT and decrease in FIB level in plasma while ATIII% showed no significant change. Decreased protein synthesis due to lack of testosterone could be the cause of our findings. Defective fibrinolysis has been reported to be frequent in hypogonadic men[23], raised plasminogen activator inhibitor (PAI) levels was suggested to be the main cause for this defective fibrinolysis[24]. Hinata et al.[25] suggested that danazol (a 2,3 isoxazol derivative of 17-alpha ethinyl testosterone) affects the turnover of vitamin K-dependent clotting factors, an impairment of synthesis being

a likely mechanism. Vitamin K-dependent coagulation factors seem to be particularly susceptible to effects of sex steroids[**26**].

To conclude, the present result confirmed the stimulatory effect of male hormones on platelet aggregability, an effect which is mainly due to increased generation of prostaglandins in platelets. Regarding blood coagulation, mesterolone treatment caused no change in PT, APTT and FIB level whereas ATIII% increased. These negative results could be due to absence of a stimulatory effect of mesterolone on coagulation factors or more likely due to an increase in the level of some coagulation proteins (not measured in the present study) which is masked by the increased ATIII%. Finally, the prolonged PT and APTT and decreased FIB in castrated rats could be explained by decrease in the synthesis of coagulation proteins due to lack of male hormones.

References

1. JOHNSON, M.; RAMEY, E. and RAMWELL, P.W. : Androgen-mediated sensitivity in platelet aggregation. Am. J. Physiol., 232 (4) : H381-H385, 1977.
2. PEARLMAN, W.H. and CREPY, O. : Steroid-protein interaction with particular reference to testosterone binding by human serum. J. Biol. Chem.; 242 : 182-189, 1967.
3. BREIER, C.; MUHLBERGER, V.; DREXEL, H. et al. : Essential role of post-heparin lipoprotein lipase activity and of plasma testosterone in coronary artery disease. Lancet, 1 : 1242-1244, 1985.
4. SEWDARSEN, M.; JIALAL, I.; VYTHILINGUM, S. et al. : Sex hormone levels in young Indian patients with myocardial infarction. Arteriosclerosis, 6 : 418-421, 1986.
5. UZUNOVA, A.D.; RAMEY, E.R. and RAMWELL, P.W. : Arachidonate-induced thrombosis in mice : Effects of gender or testosterone and estradiol administration. Prostaglandins, 13 : 995-1002, 1977.
6. NAGASAWA, H.; KIM, B.K.; STEINER, M. and BALDINI, M.G. : Inhibition of thrombin-neutralizing activity of antithrombin III by steroid hormones. Thromb. Haemostas., 47 : 157-161, 1982.
7. KLOCKING, H.P.; MARKWARDT, F. and HOFFMAN, A. : Animal experiments on the enhancement of fibrinolytic potential by anabolic steroids. Biomed. Biochem. Acta, (43) 4 : 501-507, 1984.
8. WINN, M.J. and PARK, B.K. : The effects of 17 beta-estradiol or testosterone on the response to S-warfarin in castrated male rats. J. Pharm. Pharmacol., 39 (11) : 958-960, 1987.
9. BORN, G.V.R. : Aggregation of blood platelets by adenosine diphosphate and its reversal. Nature, 194 : 927, 1962.
10. ROGER, K. and MICHAEL, C.S. : Platelet-peta-adrenoceptors. Br. J. Pharmac., 79 : 681, 1983.
11. FIRKIN, B.G. : Platelet agonists in : The platelet and its disorders edited by Barry G Firkin : MTP Press Limited, P. 58, 1985.
12. PILO, R.; AHARONY, D. and RAZ, A. : Testosterone potentiation of ionophore and ADP induced platelet aggregation : Relationship to arachidonic acid metabolism. Thromb. Haemost., (Stuttgart) 46 (2) : 538-542, 1981.
13. FLORENCE, A.T. and RAHMAN, R. : The effect of some oral contraceptive steroids on platelet electrophoretic mobility in vitro. J. Pharm. Pharmacol., 24 : 983-985, 1972.
14. TURKSOY, R.N.; PHILIPS, L.L. and SOUTHAM, A.L. : Influence of ovarian function on the fibrinolytic enzyme system. Ovulatory and anovulatory cycles. Am. J. Obstet. Gynaecol., 82 : 1211-1215, 1961.
15. CASPARY, E.A. and PEBERDY, M. : Oral contraception and blood platelet adhesiveness. Lancet, 1 : 1142-1143, 1965.

16. PEARLMAN, W.H. and CREPY, O. : Steroid-protein interaction with particular reference to testosterone binding by human serum. J. Biol. Chem., 242 : 182-189, 1967.

17. JOHNSON, M.; RAMEY, E. and RAMWELL, P.W. : Sex and age differences in human platelet aggregation. Nature, 253 : 355-357, 1975.

18. ROBEL, P.; CORPECHOT, C. and BAULIEU, E.E. : Testosterone and androstanolone in rat plasma and tissues. FEBS letters, 33 : 218-220, 1973.

19. SPRANGER, M.; ASPEY, B.S. and HARRISON, M.J. : Sex difference in antithrombotic effect of aspirin. Stroke, 20 (1) : 34-37, 1989.

20. OWENS, M.R.; CIMINO, C.D. and DONNELLY, J. : Testosterone effects on biosynthesis of coagulation proteins. Thromb. Haemost., 57 (3) : 259-262, 1987.

21. BLOMBACK, M.; ENEROTH, P.; LANDGREN, B.M. et al. : On the intraindividual and gender variability of hemostatic components. Thromb. Haemost., 67 (1) : 70-75, 1992.

22. AL-MONDHIRY, H.; MANNI, A.; OWEN, J. and GORDON, R. : Hemostatic effects of hormonal stimulation in patients with metastatic prostate cancer. Am. J. Hematol., 28 : 141-145, 1988.

23. BENNET, A.; SIÉ, P.; CARON, P. et al. : Plasma fibrinolytic activity in a group of hypogonadic men. Scand. J. Clin. Lab. Invest., 47 : 23-27, 1987.

24. CARON, P.; BENNET, A.; CAMARE, R. et al. : Plasminogen activator inhibitor in plasma is related to testosterone in men. Metabolism, 38 (10) : 1010-1015, 1989.

25. HINATA, S.; HIEKATA, T.; KAMATA, S. and MIEDA, T. : Subarachnoid hemorrhage following commencement of danazol treatment in a paticnt well controlled on warfarin anticoagulation. Kyobu-Geka, 44 (7) : 555-557, 1991.

26. JOLLY, D.W.; KADIS, B.M. and NELSON, T.E. : Estrogen and prothrombin synthesis. The protbrombinogenic effect of estrogen. Biochem. Biophys. Res. Comm., 74 : 41-49, 1977.