

# *Hemostatic System Changes Induced by 50 $\mu$ g and 30 $\mu$ g Estrogen/Progestogen Oral Contraceptives Modification of Estrogen Effects by Levonorgestrel*

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*Three oral contraceptive preparations were compared for effects on blood coagulation, fibrinolysis and platelet function in a total of 86 healthy young women. Two of the preparations contained 30 $\mu$ g ethinyl estradiol combined with levonorgestrel or norethindrone acetate, and the third contained 50 $\mu$ g mestranol and norethindrone. Tests were conducted before use of the agents, at intervals over 48 weeks' use and after discontinuation. This study showed that the changes in coagulation activity and fibrinolysis were largely related to the dose of estrogen in the contraceptive preparation; the smallest changes occurred with the low-dose (30 $\mu$ g) preparations. This study also showed that changes occurring in certain coagulation factors and inhibitors were significantly*

*smaller in the preparation containing 30 $\mu$ g of ethinyl estradiol combined with levonorgestrel, suggesting that the progestogen used in these combination oral contraceptives modifies the estrogen effects.*

## **Introduction**

Combined estrogen/progestogen oral contraceptives have been shown by epidemiologic studies, in both Europe and North America, to increase the relative risk of thromboembolic disease,<sup>1,2</sup> myocardial infarction<sup>3,4</sup> and stroke.<sup>5</sup> In general these complications have been associated with older age in users, smoking and the use of agents containing 50 $\mu$ g or more of estrogen.

Estrogen/progestogen oral contraceptives containing 50 $\mu$ g of estrogen have been shown to induce changes in certain components of the blood coagulation and fibrinolytic systems and to affect platelet function. Recent studies<sup>6,7</sup> have suggested that formulations containing less than 50 $\mu$ g of estrogen decrease the magnitude of these changes and that the use of these formulations may be associated therefore with less risk of thrombovascular complications.

New formulations of oral contraceptives have now been developed containing low doses of estrogen, usually 30 $\mu$ g or less of ethinyl estradiol, in combination with different progestogens.

The study reported below compared the effects of three estrogen/progestogen contraceptives on blood coagulation, fibrinolysis and platelet function. Two preparations containing 30 $\mu$ g of ethinyl estradiol combined with different progestogens (levonorgestrel or norethindrone acetate) were studied to determine whether estrogen effects on the coagulation system were modified by the progestogen used in combination with it. The third preparation, which contained 50 $\mu$ g mestranol and norethindrone, was studied in parallel with the others to compare the effects of 30 $\mu$ - and 50 $\mu$ -estrogen preparations.

## **Materials and Methods**

Eighty-six healthy young women who had decided to use oral contraception volunteered for the study at their six-week postnatal checkup at Rotunda Hospital, Dublin. All subjects for the study were required to meet the following qualifications: age between 18 and 30 years, proven fertility (having had at least one child), not lactating, not diabetic and with no history of gestational diabetes, no history of migraine, no history of thrombovascular disease, no abnormal

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vaginal bleeding or breast abnormality, blood pressure not exceeding 140/90 mm Hg, no history of liver or renal disease, no history of hormonal therapy for the previous three months and normal cervical cytology.

The subjects were randomly allocated to three groups. Group I comprised 31 women who were given Norinyl 1+50 (Ortho-Novum 1/50), containing 1.0 mg norethindrone and 50 $\mu$ g mestranol. Group II comprised 27 women given Loestrin 1.5/30, containing 1.5 mg norethindrone acetate and 30 $\mu$ g ethynodiol diacetate. Group III comprised 28 women given Ovranette (Nordette), containing 150 $\mu$ g levonorgestrel and 30 $\mu$ g ethynodiol diacetate.

#### *Method and Timing of Blood Collection*

The women were seen in the morning and had been asked to avoid any physical exercise beforehand. Prior to venipuncture they rested for 10 to 15 minutes. Venous blood was taken from an antecubital vein in the right arm with a minimum of venous occlusion. A sphygmomanometer was then applied around the patient's left arm and kept inflated midway between the systolic and diastolic blood pressures for ten minutes. The post-venous-occlusion blood sample was taken from the distended antecubital vein in the left arm.

#### *Laboratory Methodology*

The following laboratory tests were undertaken: prothrombin clotting time and kaolin cephalin clotting time,<sup>8</sup> factor II,<sup>9</sup> factor VII,<sup>10</sup> factor X,<sup>11</sup> antifactor Xa (anti-Xa),<sup>12</sup> antithrombin III<sup>13</sup> and fibrin plate fibrinolysis (euglobulin fraction)<sup>14,15</sup> in resting and post-venous-occlusion blood samples. Thrombin-induced platelet aggregation was measured by Chandler's tube technique.<sup>16</sup>

Prothrombin clotting time, kaolin cephalin clotting time, factor VII and the fibrin plate assays were carried out within 30 minutes of venipuncture. Factors II and X, anti-Xa and antithrombin III activities were assayed on fresh frozen plasma stored at -20°C. The platelets were counted within 30 minutes of specimen collection, and the platelet size distribution was determined within two hours.

#### *Timing of Blood Samples*

Blood specimens were taken as follows: preliminary (6 weeks or more after delivery and before hormone therapy), 6 weeks after the start of oral contraception and thereafter at 12, 24, 36 and 48 weeks. Use of the contraceptive preparations was stopped after 48

weeks, and blood specimens were taken 6 and 8 weeks after discontinuation. The results were analyzed by computer, and paired and unpaired t-tests were used to determine the level of significance of the observed changes.

#### **Results**

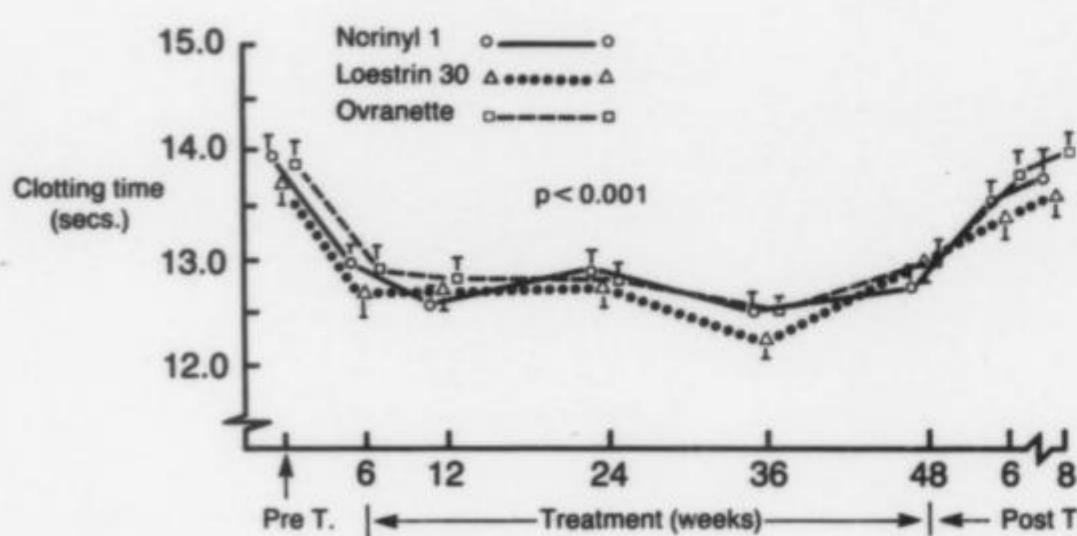
##### *Serial Changes in the Coagulation System*

**Prothrombin Time.** The prothrombin clotting time and kaolin cephalin clotting time were shortened significantly with all three preparations ( $p<0.001$ ) from the sixth week of treatment onwards. No difference was found between the groups (Figures 1 and 2). Both variables returned to the pretreatment values within six to eight weeks of discontinuation.

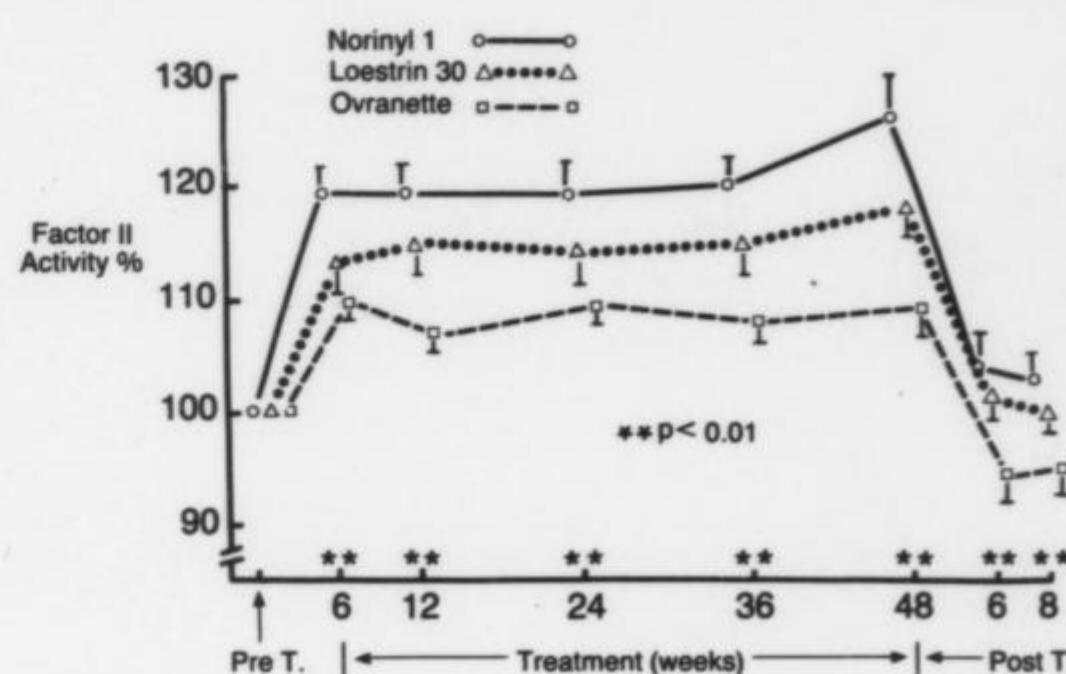
**Factor II.** In all three groups, factor II activity increased significantly ( $p<0.001$ ) from the sixth week onwards and returned to pretreatment levels within six to eight weeks of discontinuation (Figure 3). Factor II activity was significantly higher with Norinyl 1+50 than with Ovranette from the sixth week of treatment onward ( $p<0.001$ ) and higher than Loestrin 1.5/30 at 48 weeks of treatment ( $p<0.05$ ). The increase in factor II activity was higher with Loestrin 1.5/30 than with Ovranette at 12 weeks, 48 weeks ( $p<0.01$ ) and 36 weeks of treatment ( $p<0.05$ ).

**Factor VII.** The effects on factor VII of oral contraceptives containing 50 $\mu$ g or 30 $\mu$ g of estrogen are given in Figure 4. Women taking Norinyl 1+50 had a highly significant increase in factor VII activity from the sixth week of treatment onward ( $p<0.001$ ). Women taking the 30 $\mu$ g-estrogen preparations also had a significant increase in factor VII activity at 6, 12, 24 and 36 weeks of treatment ( $p<0.01$ ) but no significant change at the 48-week visit. Women taking Ovranette showed less of an increase in factor VII activity when compared with those taking Norinyl 1+50 at 24 weeks ( $p=0.001$ ) and 48 weeks ( $p<0.001$ ). A smaller increase in factor VII activity was also found in women taking Loestrin 1.5/30 at 12, 36 and 48 weeks ( $p<0.05-0.001$ ) when compared with Norinyl 1+50. No significant difference was found between Loestrin 1.5/30 and Ovranette. Factor VII activity returned to pretreatment levels within six weeks of the patients' discontinuing any of the preparations.

**Factor X.** Factor X activity increased significantly



**Figure 1**  
Effects of estrogen-containing oral contraceptives on prothrombin clotting time in three groups of women.



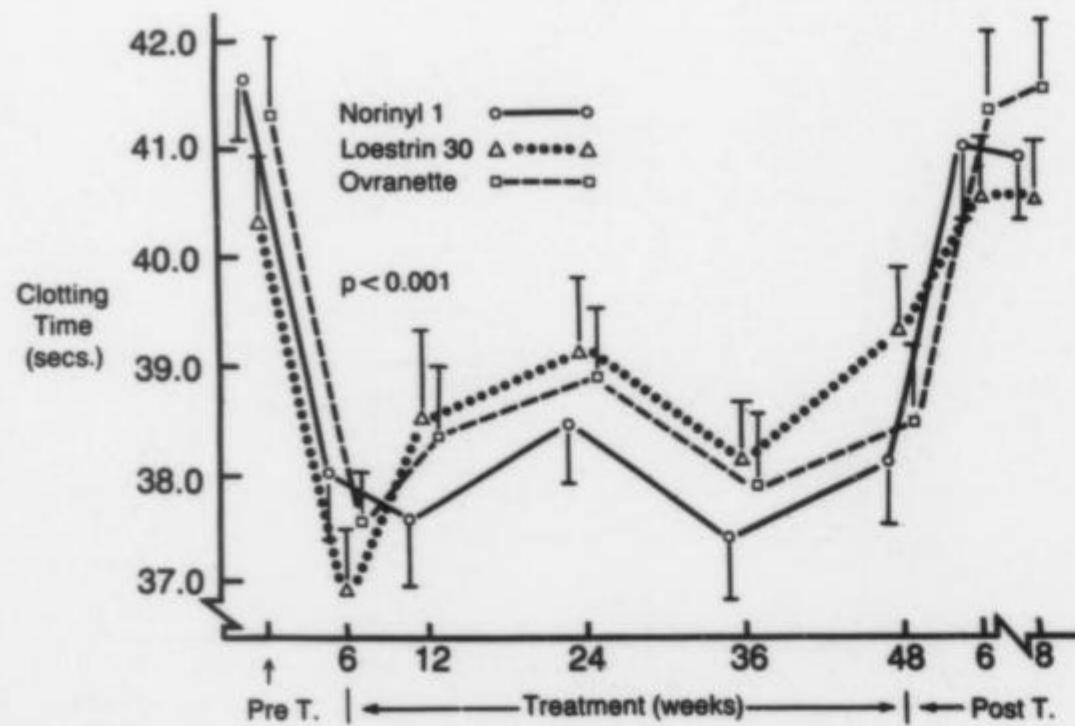
**Figure 3**  
Effects of estrogen-containing oral contraceptives on factor II activity in three groups of women.

with all three preparations from the sixth week of treatment ( $p<0.001$ ) (Figure 5). The increase in factor X activity was significantly higher with Norinyl 1+50 than with Ovranette throughout treatment ( $p<0.01-0.001$ ). No significant difference in factor X levels was found between women taking Norinyl 1+50 and Loestrin 1.5/30 or between women taking the two 30 $\mu$ g-estrogen preparations. Factor X activity was still higher than pretreatment in all three groups up to eight weeks after discontinuation of the preparations.

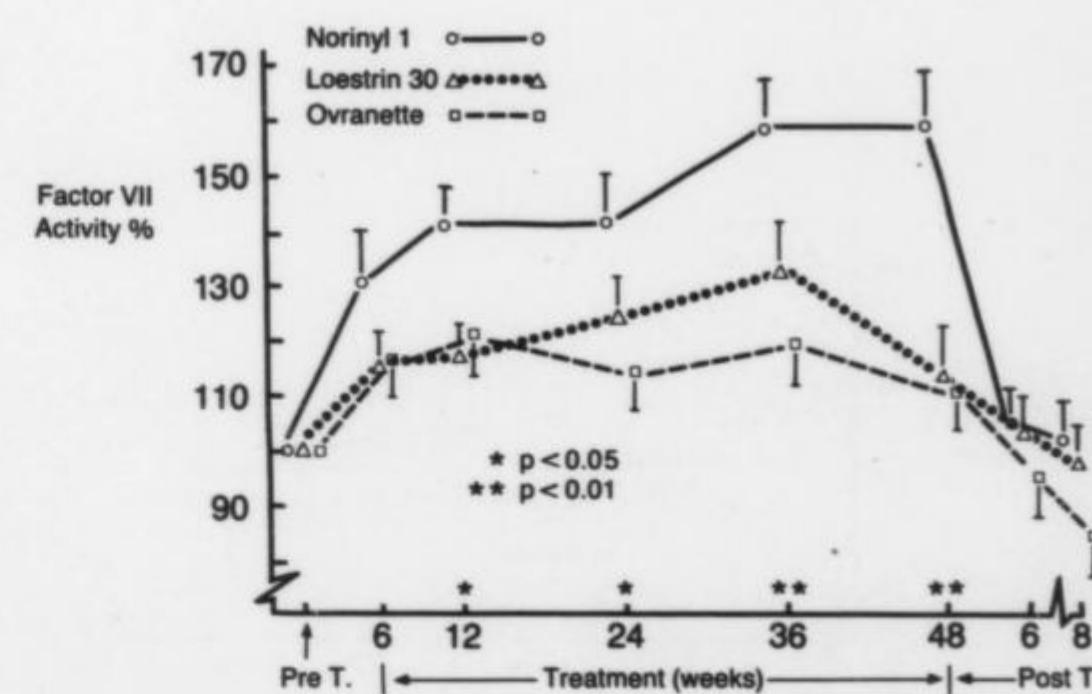
**Antithrombin III.** A significant decrease in the coagulation inhibitor antithrombin III was found with Norinyl 1+50 and Loestrin 1.5/30 from the sixth week of

treatment onward ( $p<0.001$ ) (Figure 6). In women taking Ovranette the decrease in antithrombin III was not significant except at the 48th week ( $p<0.01$ ). Antithrombin III activity was significantly lower with Norinyl 1+50 than with Loestrin 1.5/30 at 48 weeks ( $p<0.05$ ) and at 6 weeks when compared with Ovranette ( $p<0.05$ ). No significant difference in antithrombin III activity was found between Ovranette and Loestrin 1.5/30. Antithrombin III activities returned to pretreatment levels with all three preparations within six weeks of discontinuation.

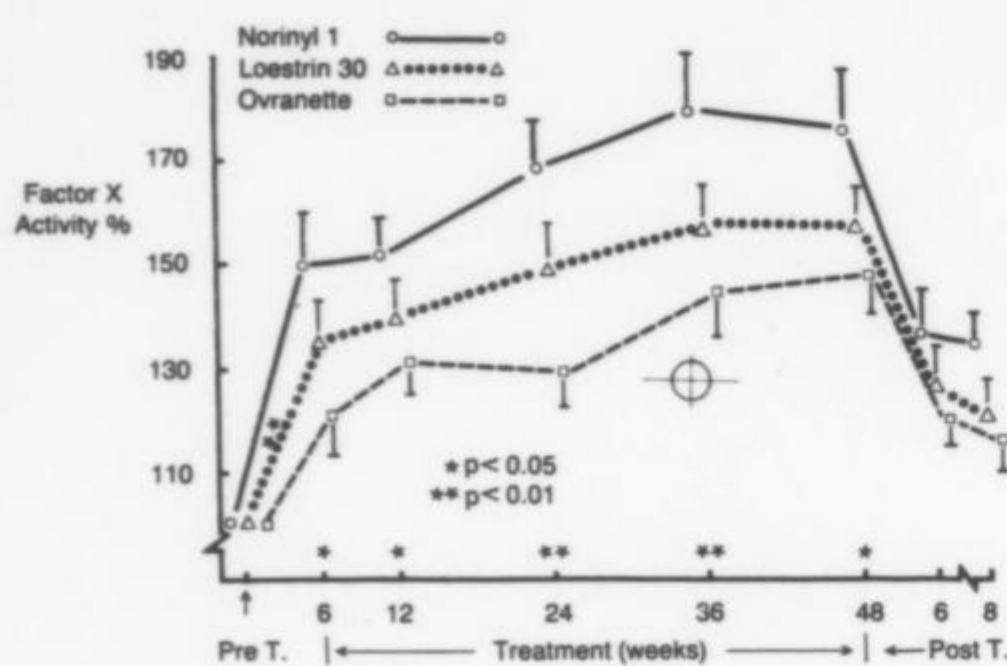
**Anti-Xa.** A significant decrease in anti-Xa activity was found with all three preparations from the sixth week of treatment on ( $p<0.001$ ) (Figure 7). Anti-Xa



**Figure 2**  
Effects of estrogen-containing oral contraceptives on kaolin cephalin clotting time in three groups of women.



**Figure 4**  
Factor VII activity in three groups of women on estrogen-containing oral contraceptives.



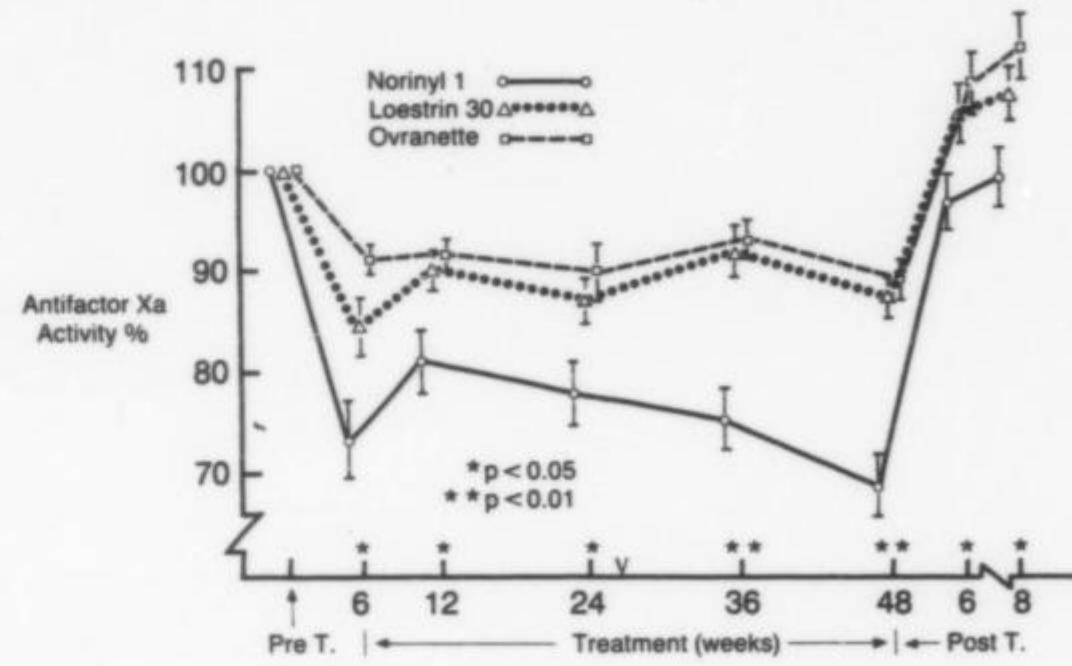
**Figure 5**  
Factor X activity in three groups of women on estrogen-containing oral contraceptives.

activity returned to the pretreatment level with Norinyl 1+50 and Loestrin 1.5/30 and to a level above the pretreatment one with Ovranette within six to eight weeks of discontinuation.

Women taking the 50 $\mu$ g-estrogen preparation showed significantly lower anti-Xa levels throughout treatment when compared with women taking the 30 $\mu$ g-estrogen preparations ( $p<0.01-0.001$ ). Anti-Xa activity was significantly lower in women taking Loestrin 1.5/30 than Ovranette at the sixth week of treatment ( $p<0.05$ ), but thereafter no significant difference was found between the two 30 $\mu$ g-estrogen preparations.

#### Serial Changes in Blood Fibrinolysis

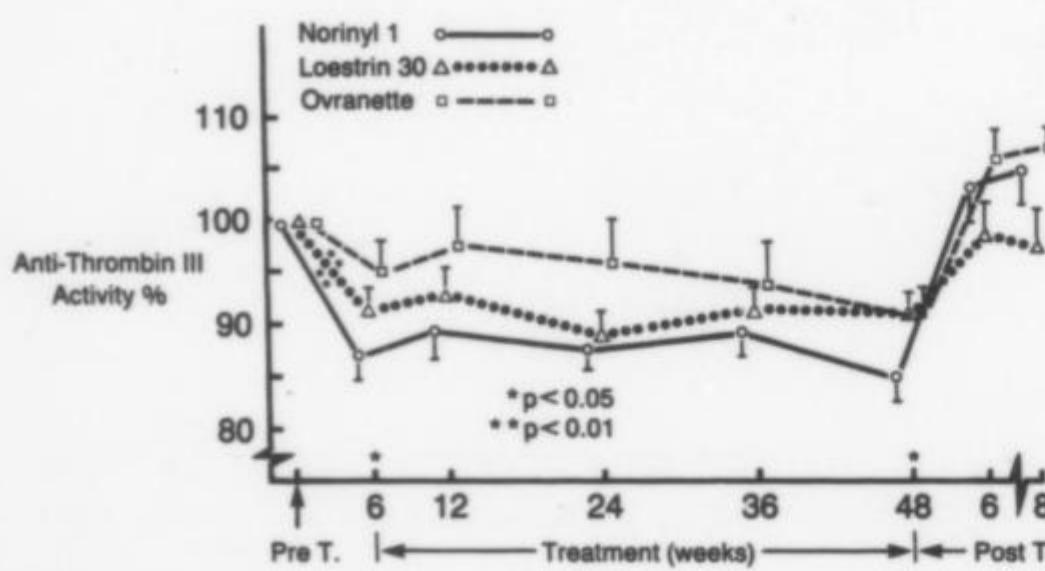
**Resting Fibrinolytic Activity.** Resting fibrinolytic ac-



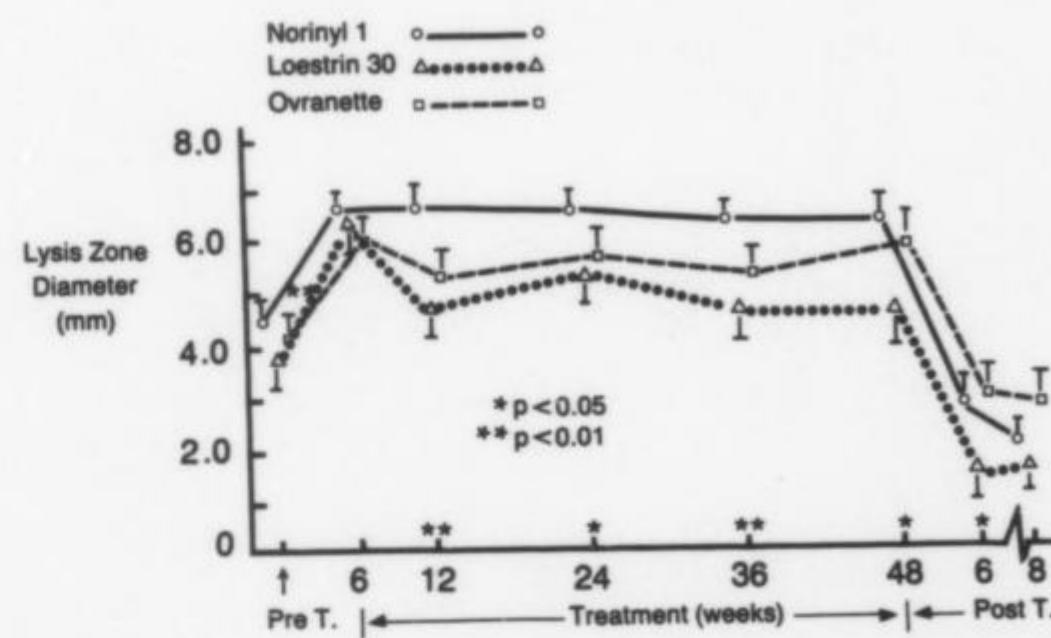
**Figure 7**  
Anti-Xa activity in three groups of women taking estrogen-containing oral contraceptives.

tivity was significantly increased during treatment in all three groups ( $p<0.05-0.001$ ) (Figure 8), but the increase in resting fibrinolysis was less with Ovranette and Loestrin 1.5/30. The fibrinolytic activity with Norinyl 1+50 was significantly higher than with Loestrin 1.5/30 from 12 weeks onward ( $p<0.05-0.001$ ). No significant difference was found between women taking Ovranette and those taking Loestrin 1.5/30.

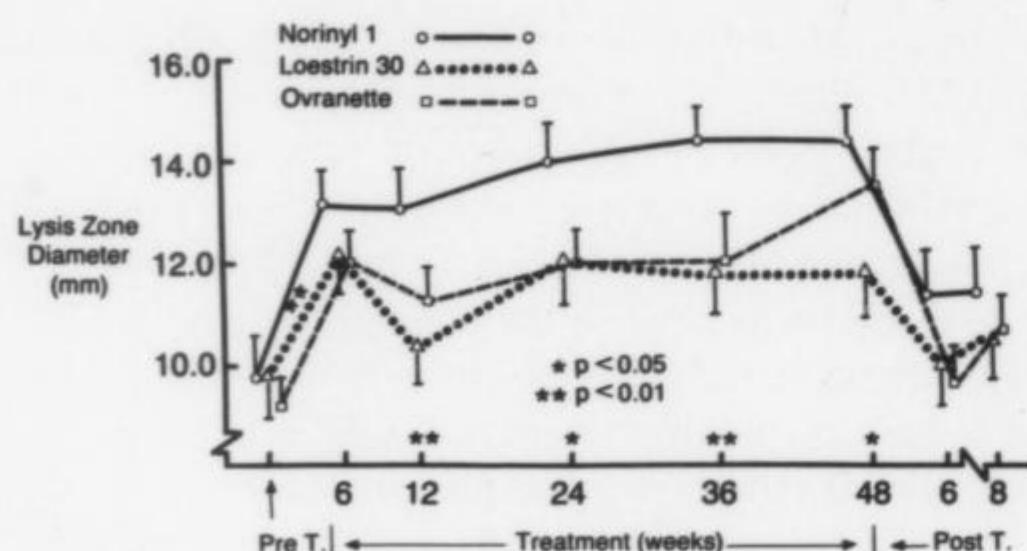
**Fibrinolytic Potential after Ten-Minute Venous Occlusion.** A highly significant increase in the fibrinolytic potential was found with Norinyl 1+50 and Ovranette after six weeks when compared with pre-treatment levels ( $p<0.01-0.001$ ) (Figure 9). The increase in fibrinolytic potential was smaller with Loestrin 1.5/30. Women taking Norinyl 1+50 showed



**Figure 6**  
Effects of estrogen-containing oral contraceptives on anti-thrombin-III activity in three groups of women.



**Figure 8**  
Effects of estrogen-containing oral contraceptives on resting fibrinolytic activity in three groups of women.

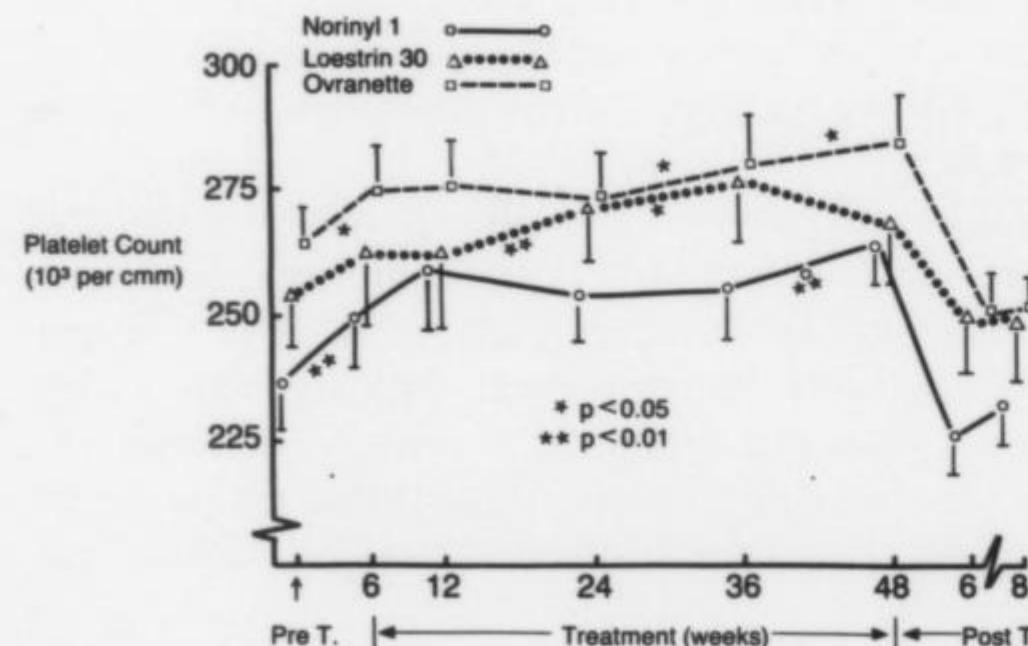


**Figure 9**  
Effects of estrogen-containing oral contraceptives on fibrinolytic potential after ten minutes' venous occlusion in three groups of women.

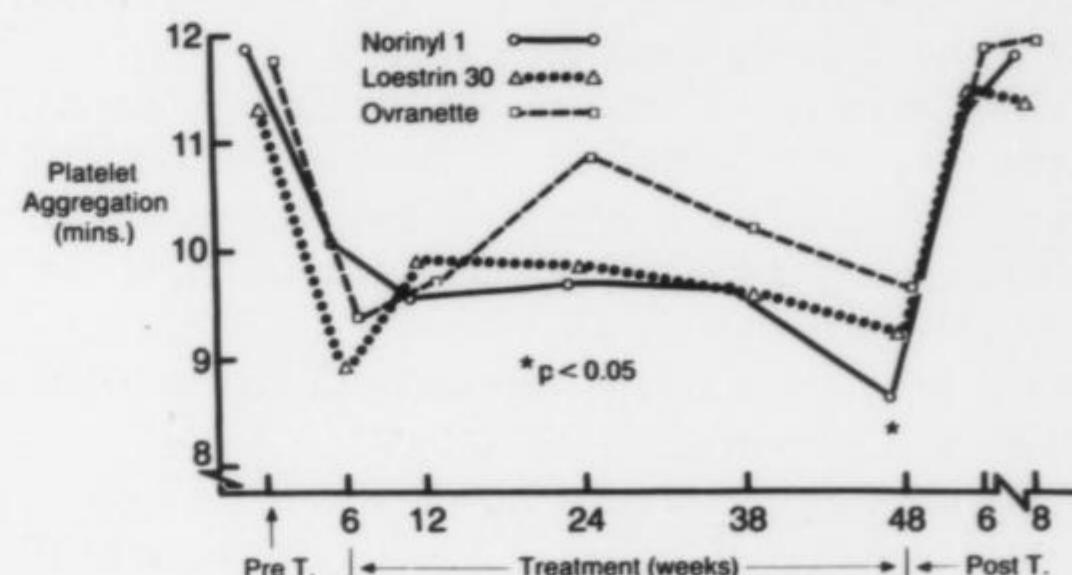
significantly higher levels than did those taking the 30 $\mu$ g preparations from 12 weeks onward ( $p<0.05-0.01$ ). No significant difference was found between women taking Ovranette and Loestrin 1.5/30.

**Serial Changes in Platelet Number, Function and Size.** In women taking Norinyl 1+50 the platelet count showed a significant increase from the sixth week onward ( $p<0.01-0.001$ ) (Figure 10). The platelet count was also significantly increased with Loestrin 1.5/30 and Ovranette from 24 weeks of treatment onward ( $p<0.05-0.01$ ). No significant difference was found between the groups, and the platelet count returned to pretreatment levels within six weeks of discontinuation.

**Spontaneous Platelet Aggregation.** Spontaneous platelet aggregation (Chandler's tube method) was



**Figure 10**  
Effects of estrogen-containing oral contraceptives on platelet number in three groups of women.



**Figure 11**  
Effects of estrogen-containing oral contraceptives on spontaneous platelet aggregation in three groups of women.

shortened significantly with all three preparations from the six-week visit onward ( $p<0.05-0.01$ ) (Figure 11). The aggregation time returned to pretreatment values within six weeks of discontinuation in all three groups. No significant difference was found between the groups except at the 48-week visit, when Norinyl 1+50 produced a significantly shortened platelet aggregation as compared with Ovranette ( $p<0.05$ ).

**Distribution of Platelet Size.** No significant difference was found in platelet size distribution in women using the three different types of oral contraceptives as compared with the distribution after discontinuation.

## Discussion

The development of a blood clot within the vascular tree involves an interaction between the blood vessel wall, platelets and the coagulation and fibrinolytic systems. Oral contraceptive preparations containing estrogen have been shown to accelerate blood clotting and platelet aggregation.

The alterations that do occur in the hemostatic system have been ascribed to the estrogen component of the oral contraceptive. The lowering of the estrogen dosage has been shown to reduce the extent of the changes in plasma fibrinogen, factor VII and anti-thrombin III.<sup>6,7</sup>

In the study reported here the prothrombin clotting time and kaolin cephalin clotting time showed a significant shortening with all three preparations from the sixth week onwards and no significant difference between the groups. Both tests showed a re-

turn to pretreatment levels within six to eight weeks of discontinuation in all three groups.

The activity of individual coagulation factors, however, showed marked differences between the three preparations. Factor II activity increased with all three preparations but was significantly higher (10–15%) in women taking Norinyl 1+50 as compared with those taking Ovranette from the sixth week of treatment ( $p<0.01-0.001$ ).

Women taking Loestrin 1.5/30 showed a significantly higher level of factor II activity as compared with those taking Ovranette ( $p<0.05-0.01$ ). This finding suggests that the increase in the factor II level depended not only on the dose of estrogen but also on the type of progestogen used in combination with it.

Factor VII activity increased steadily in women taking Norinyl 1+50 and reached almost 160% at the 36th–48th week of treatment. Women taking the 30 $\mu$ g-estrogen preparation showed a less steep rise in factor VII activity. Significantly lower levels of factor VII were found in women taking Ovranette as compared with those taking Norinyl 1+50. Less of an increase in factor VII activity was also found in women taking Loestrin 1.5/30, and no significant difference was found between those taking Loestrin 1.5/30 and Ovranette.

A gradual increase in factor X activity was found with all three preparations from the sixth week of treatment onward. By the 40th week of treatment, factor X activity had increased by 80% with Norinyl 1+50, 50% with Loestrin 1.5/30 and 48% with Ovranette. The factor X levels decreased after pill discontinuation but had not reached pretreatment levels by eight weeks after treatment. No significant difference in factor X levels was found in women using Norinyl 1+50 as compared with those using Loestrin 1.5/30. However, factor X activity with Norinyl 1+50 was significantly higher as compared with Ovranette from the 12th week of treatment onward ( $p<0.05-0.01$ ).

Antithrombin III activity is considered to be one of the most important parameters for measuring any tendency toward thrombosis. In the present study a highly significant decrease in antithrombin III activity ( $p<0.01-0.001$ ) was found from the sixth week of treatment in women taking Norinyl 1+50 and Loestrin 1.5/30. The depression of anti-thrombin III with Ovranette was much less and did not reach statistical significance until the 48th week of treatment, at which time the level had decreased by 10%. At the sixth week of treatment the group taking Norinyl

1+50 showed a 15% depression of anti-thrombin III as compared with 5% in those taking Ovranette ( $p<0.05$ ). Similarly, in women taking Norinyl 1+50 the decrease in anti-Xa activity was significantly greater than in women taking the 30 $\mu$ g-estrogen preparations. Anti-Xa activity was decreased by 20–30% throughout treatment with Norinyl 1+50. The decrease in anti-Xa activity with Loestrin 1.5/30 was 8–15% during treatment ( $p<0.001$ ). In the group taking Ovranette the decrease in anti-Xa activity was much smaller than that occurring in the other groups, and a decrease of 10% did not occur until 48 weeks of treatment.

Resting fibrinolytic activity increased significantly with all three preparations and significantly more in the group given the 50 $\mu$ g-estrogen preparation than in those given the 30 $\mu$ g preparations. Post-venous-occlusion fibrinolysis was also increased significantly in all three groups and was significantly higher with Norinyl 1+50 ( $p<0.05-0.01$ ). These findings provide clear evidence of an increase in resting fibrinolytic activity and in fibrinolytic potential in women using different types of combined oral contraceptives. This increase appears to be dependent on the estrogen dosage; the duration of treatment and the progestogen component do not seem to have any substantial effect. This finding suggests that in women taking oral contraceptives the increase in coagulation activity may be counterbalanced to a large extent by an increased fibrinolytic response, thus protecting the dynamic balance between coagulation and fibrinolysis.

### Conclusion

The changes in coagulation activity and fibrinolysis induced by oral contraceptives are largely related to the estrogen dosage. Simple screening tests, such as the prothrombin and kaolin cephalin clotting times, show a response with all three preparations but are not sensitive enough to show differences between the groups.

This study showed that the changes occurring in certain coagulation factors and inhibitors were significantly smaller in women taking 30 $\mu$ g of estrogen combined with levonorgestrel than in those taking 30 $\mu$ g of estrogen combined with norethindrone acetate. This finding strongly suggests that the progestogen used in combination modifies the effect of the estrogen.

Current research is concerned with finding estrogen/progestogen combinations that produce the smallest changes in the hemostatic system and in other aspects of metabolism. From this point of view

the combination of 30 $\mu$ g of estrogen with 150 $\mu$ g of levonorgestrel is a major step forward. Since this combination is associated with the smallest changes in the hemostatic system, it is to be expected that its use will be associated with a further lowering of the risk of thrombovascular complications.

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