Fibrinolytic Enzyme System and Pregnancy

JOHN BONNAR,* M.B., M.R.C.O.G. ; G. P. McNICOL,† M.D., PH.D., F.R.C.P.(ED., GLASG.)
A. S. DOUGLAS,‡ M.D., F.R.C.P.(LOND., ED., GLASG.)

Summary : The effect of pregnancy on the components of the fibrinolytic enzyme system was determined by serial observations on 10 healthy women during normal pregnancy, labour, and the puerperium. The plasminogen level was substantially increased in the third trimester ; the increase occurred pari passu with a pronounced increase in fibrinogen concentration. After allowing for the expansion of plasma volume in pregnancy a twofold increase in the absolute amounts of circulating fibrinogen and plasminogen was found. In late pregnancy and during labour the level of plasminogen activator was greatly decreased, whereas a normal or increased level was present in the first week of the puerperium. No alteration in the levels of inhibitors of plasminogen activation by urokinase was found during normal pregnancy. The thrombin time and platelet count remained unchanged during pregnancy and labour but the platelet count rose significantly during the first week of the puerperium.

The haemostatic mechanism in pregnancy appears to be altered towards an enhanced capacity to form fibrin and a diminished ability to lyse fibrin. These changes may be a physiological development to ensure the integrity of the foetal and maternal circulations and provide rapid and effective haemostasis in the uterus during and after placental separation. Nevertheless, the changes may also establish a vulnerable state for intravascular fibrin deposition.

Introduction

The fibrinolytic enzyme system or plasminogen-plasmin system has a physiological role complementary to the coagulation mechanism in maintaining the patency of the blood vessels by promoting the removal of intravascular fibrin. The behaviour of the fibrinolytic mechanism in human pregnancy is therefore of special interest because an increase in the concentration of certain coagulation factors, particularly fibrinogen, is known to occur as pregnancy advances. Fibrinolytic activity has been reported to be decreased in late pregnancy, but many of the previous reports are single observations obtained under varying conditions and conflicting results have been presented. The purpose of this investigation was to elucidate the changes in the components of the fibrinolytic enzyme system induced by pregnancy and parturition by serial observations on a group of healthy women followed throughout normal pregnancy, labour, and the puerperium.

According to current concepts the coagulation and fibrinolytic systems may be in a state of dynamic equilibrium which keeps the vascular compartment intact and patent, the coagulation system laying down fibrin on the vascular endothelium to seal any gaps which may occur, and the fibrinolytic system removing such fibrinous deposits after they have served their

haemostatic function. The fibrinolytic system has a similar basic structure to the coagulation system ; plasminogen, an inactive plasma globulin, is converted by activators to plasmin, a proteolytic enzyme which digests fibrin, with the release of soluble products (Fig. 1). Plasminogen activators are present in many tissues and plasminogen activator in the plasma appears to be responsible for the physiological fibrinolytic activity in blood.
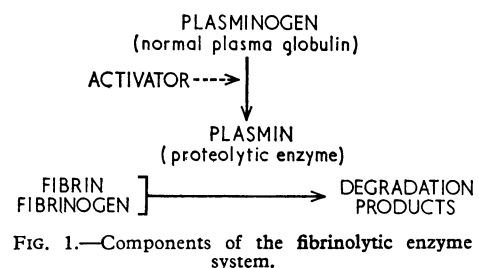

FIG. 1.—Components of the fibrinolytic enzyme system.

Patients and Methods

Ten healthy women with uncomplicated pregnancies were serially studied with their full, informed consent from the first trimester to term and during labour and the puerperium. In addition, a further 30 women were investigated six to eight weeks after normal confinement to obtain reference data for the statistical evaluation of the alterations of components of the fibrinolytic enzyme system during pregnancy. Venous blood samples were taken with the minimum of venous occlusion, and tests of fibrinolytic activity were invariably carried out within one hour of collection of the blood, which was kept at 4° C. The following assays were performed.

Fibrinogen Assay.—The biochemical method of Ratnoff and Menzie (1965) was used.

Plasminogen Assay.—The method of Remmert and Cohen (1949) as modified by Alkjaersig et al. (1959) was used.

Euglobulin Lysis Time (Nilsson and Olow, 1962).—In this test fibrinogen, plasminogen, and plasminogen activators are precipitated from the plasma ; antiplasmin is discarded with the supernatant and the precipitate resuspended ; and after clotting with thrombin the lysis time of the clot at 37° C. is measured. In this investigation clot lysis has latterly been measured by automatic recording of the optical density of the clot (Cash and Leask, 1967). Lysis times and blood fibrinolytic activity are inversely related, hence a long lysis time reflects low fibrinolytic activity and a short lysis time high fibrinolytic activity. On a double logarithmic plot lysis times and activity are linearly related, and with the use of such a plot lysis times can be expressed in arbitrary units of activity assigning 1 unit a lysis time of 300 minutes (Sherry et al., 1959).

Urokinase Sensitivity Test (McNicol et al., 1963).—In this test the lysis time of a plasma clot in the presence of a standard amount of urokinase is measured. In the presence of adequate amounts of fibrinogen and plasminogen a prolonged lysis time reflects a high level of inhibitor of fibrinolysis.

Thrombin Clotting-time.—The method described by Fletcher et al. (1959) was used.

* Senior Registrar in Obstetrics and Gynaecology, Royal Maternity Hospital and Victoria Infirmary, Glasgow.
† Senior Lecturer in Medicine, University Department of Medicine, Royal Infirmary, Glasgow C.4.
‡ Professor of Medicine, University Department of Medicine, Royal Infirmary, Glasgow C.4.

Platelet Count.—Venous blood was used and a direct count performed according to the method of Dacie (1963).

Results

Fibrinogen.—The previously documented increase of plasma fibrinogen during pregnancy was confirmed (Fig. 2). The mean fibrinogen level was slightly increased in early pregnancy at 340 mg./100 ml., compared with a mean level of 285 mg./100 ml. found six weeks after delivery. The fibrinogen level gradually increased with the duration of pregnancy to a mean value of 450 mg./100 ml. at term. The rise of fibrinogen during pregnancy showed a significant positive correlation with the period of gestation ($r = +0.673$, $P < 0.001$). The levels of fibrinogen remained virtually unchanged during labour and decreased slightly during the first week of the puerperium. At the sixth postnatal week the fibrinogen levels were in the normal range for non-pregnant women.
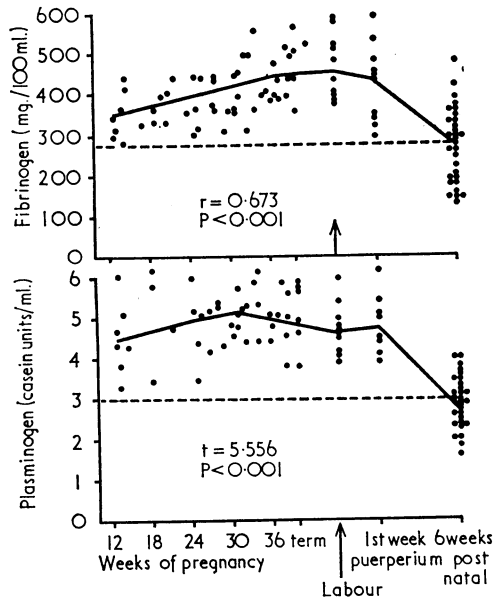

FIG. 2.—Effect of pregnancy on the levels of fibrinogen and plasminogen.

Plasminogen.—Levels of plasminogen followed serially through pregnancy are shown in Fig. 2. Raised levels of plasminogen were found as early as the fourth month of pregnancy, and from the fifth month onwards the mean value was about 5 casein units/ml. The increased level of plasminogen during pregnancy compared with the levels found six weeks after delivery was highly significant ($t = 5.556$,
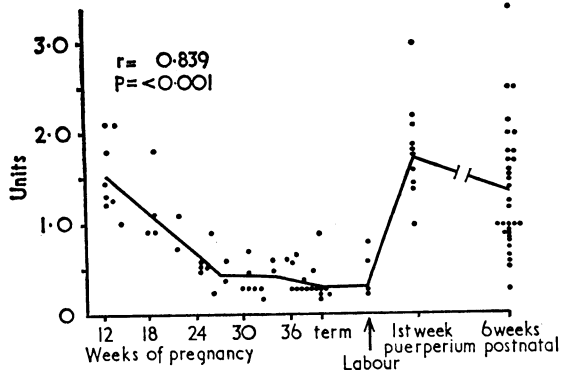
$P < 0.001$). A slight decrease of the mean plasminogen level was found during labour. In the first week of the puerperium the plasminogen levels remained raised, but by the sixth postnatal week the levels were in the normal non-pregnant range, the mean level being 2·83 casein units/ml.

Euglobulin Lysis Time.—A pronounced decrease of fibrinolytic activity during pregnancy was found, the euglobulin lysis time increasing steeply after the first trimester. The reduction in fibrinolytic activity was most pronounced in the third trimester and persisted throughout the first and second stages of labour. In the first week of the puerperium normal or slightly increased fibrinolytic activity was found. When the lysis times are expressed as units of activity, calculated as stated previously, the reduction of activator levels has a significant negative correlation with the period of gestation ($r = -0.839$, $P < 0.001$) (Fig. 3).

Urokinase Sensitivity Test.—In the present study normal pregnancy did not appear to be associated with any significant change in the level of inhibitors of plasminogen activation by urokinase.

Thrombin Time.—No significant alteration of thrombin time was found in relation to pregnancy, labour, and the puerperium.

Platelet Count.—The mean platelet count was slightly lower during pregnancy than the six-week postnatal level, and the lowest level was recorded during labour. A significant rise of the platelet count was found in the first week of the puerperium as compared with the level found in labour ($t = 2.4$, $P < 0.02$) (Fig. 4).

Discussion

An increase in the concentration of plasma fibrinogen and factors VII, VIII, and X in association with pregnancy has been reported (Pechet and Alexander, 1961 ; Kasper *et al.* 1964 ; Talbert and Langdell, 1964), and the findings in this study have shown that a considerable deviation from the normal occurs in the components of the fibrinolytic enzyme system.

The increase in the concentration of plasma fibrinogen during pregnancy is well documented, but few reports are available on plasminogen levels during pregnancy. Shaper *et al.* (1965) and Brakman (1966) found no difference between plasminogen levels in non-pregnant and pregnant women. Shaper's report was on the levels found in 10 African women, and Brakman used a different method of plasminogen assay, rendering comparisons difficult. The results reported here of clearly increased levels of plasminogen during pregnancy are in agreement with the findings of Mitchell and Cope (1965) and Nilsson and Kullander (1967). The increase of plasminogen as shown in Fig. 2 appears to occur pari passu with that of fibrinogen, and the rise above normal levels in the third
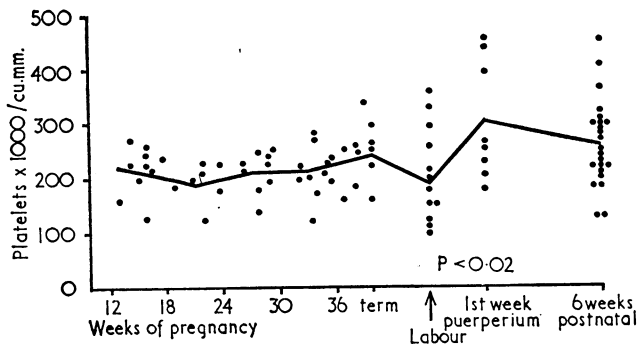

FIG. 3

FIG. 4

FIG. 3.—Level of plasminogen activator during pregnancy, labour, and the puerperium. FIG. 4.—Platelet levels during pregnancy, labour, and the puerperium.

trimester is of the order of 66% in both fibrinogen and plasminogen. Allowing for the expansion of plasma volume in pregnancy, this represents a twofold increase in the absolute amounts of circulating fibrinogen and plasminogen. By the sixth postnatal week the levels of fibrinogen and plasminogen have returned to normal. The concomitant increase of plasminogen and fibrinogen during pregnancy would appear to be consistent with the concept of a dynamic equilibrium between clotting and lysis, as a rise in the levels of these two components might be predicted to influence the haemostatic balance in opposite directions.

Decreased fibrinolytic activity during late pregnancy was first reported by Biezenski and Moore (1958) and confirmed by Shaper *et al.* (1965). The euglobulin lysis time depends on the level of fibrinogen, plasminogen, and plasminogen activator. In the presence of adequate levels of fibrinogen and plasminogen the change in the euglobulin lysis time reflects an alteration of the level of circulating plasminogen activator. The data presented therefore suggest that the decreased fibrinolytic activity observed during pregnancy represents a decrease in the level of circulating plasminogen activator, as though the plasma fibrinogen level is raised the plasminogen concentration is raised to a proportionate extent. The mechanism responsible for the decrease of effective levels of plasminogen activator is unknown. Physical exercise and mental stress in the non-pregnant are associated with a steep rise of activator levels (Sherry *et al.*, 1959). The mental and physical exertion of labour, however, surprisingly appears to provoke no rise of fibrinolytic activity, and the low level of fibrinolytic activity persists despite the strenuous efforts and agitation of the second stage of labour.

In the present study pregnancy did not appear to be associated with any significant change in the levels of specific inhibitors to plasminogen activation by urokinase. A selective increase in the capacity of the blood of pregnant women to inhibit urokinase or urokinase-induced fibrinolysis was reported by Brakman and Astrup (1963), who used a different test system (the fibrin plate method) which could be influenced by other factors—for example, differing diffusion rates of activators, enzymes, and inhibitors. Using a clot lysis method Nilsson and Kullander (1967) have also reported no significant change in the level of urokinase inhibitor during pregnancy.

We found no significant alteration in the platelet count during pregnancy, apart from the sharp increase in the level of circulating platelets in the first week of the puerperium. The platelet count during pregnancy has been the subject of conflicting reports; a continuous increase in the platelet count during pregnancy was reported by Mor *et al.* (1960), and Shaper *et al.* (1968) recorded a progressive decrease as pregnancy advanced.

The complex changes involving both coagulation and fibrinolysis which accompany pregnancy are difficult to interpret in terms of physiological significance. The control of haemorrhage is one of the obvious functions of blood coagulation, while that of fibrinolysis is the removal of fibrin formed in blood vessels and elsewhere. The two systems appear to be designed for local action, and normal pregnancy seems to alter the haemostatic balance towards an enhanced capacity to form fibrin and a diminished ability to lyse fibrin. A possible interpretation of the finding is that the alterations may be a protective mechanism designed to maintain the integrity of both the maternal and the foetal circulation during pregnancy and to provide a rapid and effective control of maternal bleeding during and after separation of the placenta. Such alterations, however, may also establish a vulnerable state for intravascular clotting and thromboembolic complications.

The hypothesis of transient coagulation in the blood which can obstruct the microcirculation is a relatively new concept in the aetiology of disease (Hardaway, 1966). In late pregnancy certain syndromes occur where intravascular fibrin deposition appears to be a feature and the inhibition of fibrinolytic activity may therefore be an important aetiological factor. These include complications such as acute tubular necrosis, Sheehan's syndrome, and the Shwartzman reaction. The recent studies on cerebral strokes in pregnancy (Jennett and Cross, 1967 ; Cross *et al.*, 1968) have shown that thrombotic occlusion of the cerebral arteries has a much higher incidence in pregnant women and results in a mortality three times higher than is found in non-pregnant women of the same age group.

Although the mechanisms whereby the alterations in coagulation and fibrinolysis are brought about in pregnancy are still obscure, knowledge of changes induced by normal pregnancy may help in elucidating the pathogenesis of those obstetric complications which may be associated with intravascular coagulation and haemorrhage.

REFERENCES

Alkjaersig, N., Fletcher, A. P., and Sherry, S. (1959). *Journal of Clinical Investigation*, 38, 1086.
Biezenski, J. J., and Moore, H. C. (1958). *Journal of Clinical Pathology*, 11, 306.
Brakman, P. (1966). *American Journal of Obstetrics and Gynecology*, 94, 14.
Brakman, P., and Astrup, T. (1963). *Scandinavian Journal of Clinical and Laboratory Investigation*, 15, 603.
Cash, J. D., and Leask, E. (1967). *Journal of Clinical Pathology*, 20, 209.
Cross, J. N., Castro, P. O., and Jennett, W. B. (1968). *British Medical Journal*, 3, 214.
Dacie, J. V. (1963). *Practical Haematology*, p. 61. London, Churchill.
Fletcher, A. P., Alkjaersig, N., and Sherry, S. (1959). *Journal of Clinical Investigation*, 38, 1096.
Hardaway, R. M. (1966). *Syndromes of Disseminated Intravascular Coagulation*. Springfield, Illinois, Thomas.
Jennett, W. B., and Cross, J. N. (1967). *Lancet*, 1, 1019.
Kasper, C. K., Hoag, M. S., Aggeler, P. M., and Stone, S. (1964). *Obstetrics and Gynecology*, 24, 242.
McNicol, G. P., Gale, S. B., and Douglas, A. S. (1963). *British Medical Journal*, 1, 909.
Mitchell, P., and Cope, I. (1965). *Clinica Chimica Acta*, 11, 414.
Mor, A., Yang, W., Schwartz, A., and Jones, W. C. (1960). *Obstetrics and Gynecology*, 16, 338.
Nilsson, I. M., and Kullander, S. (1967). *Acta Obstetricia et Gynecologica Scandinavica*, 46, 273.
Nilsson, I. M., and Olow, B. (1962). *Acta Chirurgica Scandinavica*, 123, 247.
Pechet, L., and Alexander, B. (1961). *New England Journal of Medicine*, 265, 1093.
Ratnoff, O. D., and Menzie, C. (1965). *Blood Coagulation, Hemorrhage and Thrombosis*, edited by L. M. Tocantins and L. A. Kazal, p. 224. New York, Grune and Stratton.
Remmert, L. F., and Cohen, P. P. (1949). *Journal of Biological Chemistry*, 181, 431.
Shaper, A. G., Kear, J., MacIntosh, D. M., Kyobe, J., and Njama, D. (1968). *Journal of Obstetrics and Gynaecology of the British Commonwealth*, 75, 433.
Shaper, A. G., MacIntosh, D. M., Evans, C. M., and Kyobe, J. (1965). *Lancet*, 2, 706.
Sherry, S., Fletcher, A. P., and Alkjaersig, N. (1959). *Physiological Review*, 39, 343.
Talbert, L. M., and Langdell, R. D. (1964). *American Journal of Obstetrics and Gynecology*, 90, 44.