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THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN NORMAL AND ABNORMAL PREGNANCY

A dissertation submitted for the degree of

Doctor of Medicine

of the University of Glasgow

by

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PREFACE

The studies presented in this thesis were carried out in the University Department of Medicine, Glasgow Royal Infirmary, and completed in the Nuffield Department of Obstetrics, Oxford University. The work was commenced when I was registrar at the Victoria Infirmary of Glasgow and continued while I was senior registrar at Glasgow Royal Maternity Hospital and held the Samuel Research Scholarship of the Royal College of Obstetricians and Gynaecologists. The progress of the work and its development were discussed with Professor A.S. Douglas and Dr. G.P. McNicol in association with whom it was published. The planning of the research projects, the clinical management and laboratory investigation were my own responsibility.

Colleagues who collaborated in the studies included Dr. J.F. Davidson, Dr. C.R.M. Prentice, Mrs. Christine Pidgeon, Mrs. Allison Sandiford and Mrs. Eileen Cunningham. Much of the data in the thesis has already been published or has been accepted for publication as shown below.

Publications :

1. The Fibrinolytic Enzyme System and Pregnancy.
Bonnar, J., McNicol, G. P. and Douglas, A. S.
British Medical Journal, 1969, 3, 387-389.

2. Fibrin Degradation Products in Normal and Abnormal Pregnancy and Parturition.
Bonnar, J., Davidson, J. F., Pidgeon, C. F., McNicol, G. P. and Douglas, A. S.
British Medical Journal, 1969, 3, 137-140.

3. The Behaviour of the Coagulation and Fibrinolytic Mechanisms in Abruptio Placentae.
Bonnar, J., McNicol, G. P. and Douglas, A. S.
Journal of Obstetrics and Gynaecology of the British Commonwealth, 1969, 76, 799-805.

4. The Coagulation and Fibrinolytic Mechanisms During and After Normal Childbirth.
Bonnar, J., McNicol, G. P. and Douglas, A. S.
British Medical Journal, 1970, 2, 200-203.

5. The Haemostatic Mechanism in the Uterine Circulation during Placental Separation.
Bonnar, J., Prentice, C. R. M., McNicol, G. P. and Douglas, A. S.
British Medical Journal, 1970, 2, 564-567.

6. The Physiology of the Haemostatic Mechanism during and after Childbirth.
Bonnar, J., Prentice, C. R. M., McNicol, G. P. and Douglas, A. S.
Proceedings of the Sixth World Congress of Obstetrics and Gynaecology, New York, April 1970.

7. The Coagulation and Fibrinolytic Systems in the Newborn.
Bonnar, J., McNicol, G. P., and Douglas, A. S.,
Lancet (1970) In Press.

8. The Coagulation and Fibrinolytic Systems in Pre-eclampsia and Eclampsia.
Bonnar, J., McNicol, C. P., and Douglas, A. S.
British Medical Journal (1970). In Press.

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SECTION 1

INTRODUCTORY

Chapter 1 Introduction

Chapter 2 Blood coagulation and fibrinolysis in pregnancy

Chapter 3 Current concepts of the components and functions of the coagulation and fibrinolytic mechanisms

CHAPTER 1

INTRODUCTION

My personal interest in the mechanisms of haemostasis was aroused by frequent involvement as an obstetric house officer and registrar with haemorrhagic complications of pregnancy and parturition which from time to time resulted in death of the patient. I discussed my interest in this field with Professor R.G. Macfarlane and on his invitation spent two weeks at the Medical Research Council Blood Coagulation Research Unit in Oxford. The opportunity of doing research work in pregnancy was given to me by Professor A.S. Douglas and from 1964 to 1967 I was freed of clinical duties for one day per week to work in the University Department of Medicine, Glasgow Royal Infirmary. The award of a Samuel Research Scholarship by the Royal College of Obstetrics and Gynaecologists in May, 1967 enabled me to spend my time equally, in alternate weeks, between clinical obstetrics and gynaecology and investigative work in Professor A.S. Douglas's Research Laboratory.

My initial research project was to study the effect of normal pregnancy on the fibrinolytic enzyme system and investigate the

effect of normal parturition on the coagulation and fibrinolytic systems. With this knowledge available the behaviour of the clotting and fibrinolytic mechanisms was studied in certain complications of pregnancy, particularly abruptio placentae, pre-eclampsia and eclampsia. This work is the subject of the thesis.

The thesis begins with a brief historical review of the development of knowledge of the effect of pregnancy on the haemostatic mechanism and an account of current concepts of the components and functions of the fibrinolytic enzyme system and the coagulation mechanism. After the introductory section the thesis presents the results of a serial study of the components of the fibrinolytic enzyme system in healthy women during normal pregnancy, labour and the puerperium. A marked deviation from the findings in normal non-pregnant subjects is shown to develop during pregnancy. Fibrinolytic activity in the peripheral blood remained diminished during labour but levels of fibrin breakdown products in the serum increased indicating that lysis of fibrin was probably occurring in localised areas of the vascular compartment.

To define further the effect of childbirth on the haemostatic mechanism, a detailed study of the coagulation and fibrinolytic systems was undertaken in the second stage of labour, immediately before and after delivery of the baby, in the cord blood, and immediately after separation of the placenta. As the haemostatic mechanism would seem to be designed for local action the behaviour of the clotting and fibrinolytic systems in the uterine circulation was studied during and after separation of the placenta at delivery by caesarean section. The process of placental separation was shown to be accompanied by a striking local activation of the clotting mechanism in the uterine circulation which was barely detectable in venous blood taken from the arm.

The thesis then goes on to describe the findings in the coagulation and fibrinolytic systems in abruptio placentae and evidence is produced which indicates that intravascular clotting takes place in this complication of pregnancy and that the fibrinolytic enzyme system is activated simultaneously to remove intravascular fibrin. Extremely high levels of break-down products of fibrin were found in the presence of

diminished systemic fibrinolytic activity. Fibrin breakdown products seriously disrupt both the clotting process and platelet function and their presence is likely to be a major factor in the defective haemostasis of abruptio placentae. The results of this investigation indicate that the transfusion of fresh whole blood and early vaginal delivery are the most effective ways of restoring the haemostatic mechanism to normal and that the administration of fibrinolytic inhibitors may be hazardous.

In patients with pre-eclampsia and eclampsia very low levels of plasminogen activator were found and the plasma clots showed a greater resistance to lysis by urokinase than was present in normal pregnancy. The findings in the patients with eclampsia were in keeping with an acute episode of intravascular fibrin deposition having preceded the onset of the convulsions. Fibrin deposition in the vessels at the placental site is known to be a feature of pre-eclampsia (Dixon and Robertson, 1958). An aberration of the fibrinolytic mechanism in pregnancy resulting in intravascular fibrin deposition could be of considerable aetiological importance in the syndrome of pre-eclampsia and the impaired placental function which occurs

in this complication. Further knowledge in this area may possibly define a use for fibrinolytic agents and open up a new field of rational treatment.

Plan of the thesis

The thesis is presented in two volumes; Volume I contains the text and references, and Volume II figures, tables, and an account of standard methods.

CHAPTER 2

BLOOD COAGULATION AND FIBRINOLYSIS IN PREGNANCY

From all accounts, haemorrhage from the uterus during childbirth has always been, and still remains, a major cause of maternal death. Smellie (1756) writing on postpartum haemorrhage records that "this hazardous haemorrhage is known by the violence of the discharge, wetting fresh cloths as fast as they can be applied; from the pulse becoming low and weak, and the countenance turning pale; then the extremities grow cold, she sinks into faintings, and, if the discharge is not speedily stopped or diminished, is seized with convulsions, which often terminate in death". Hewson (1771) reported on a patient at the British Lying-In Hospital who had incoagulable blood following delivery and he noted that, on heating, the blood behaved like serum and was probably "without the coagulable lymph". Despite this observation, obstetric haemorrhage and defective blood coagulation were not associated until De Lee (1901) vividly described a case of fatal haemorrhage associated with abruptio placentae and a nearly fatal haemorrhage following the delivery of a stillborn macerated foetus; in both cases the blood failed

to clot and De Lee concluded "that there are alterations of the blood or blood vessels, of a temporary nature, which prevent its clotting and thus during labour or operations cause death". De Lee suggested syphilis, malaria, or a blood disease as possible causes. Willson (1922) described a patient with "uteroplacental apoplexy" with irreversible shock and death following caesarean section and he postulated "a toxin in the nature of a haemorrhagin" which caused severe uterine haemorrhage and also affected other areas of the body.

Dieckmann (1936) reported two patients with severe abruptio placentae who had bleeding from the gums and into subcutaneous tissues and where the blood failed to clot in a test tube. By further studies he was the first to show that the plasma fibrinogen levels were abnormally low in patients with severe abruptio placentae. Dieckmann suggested that massive retroplacental haemorrhage might deplete the circulating blood of fibrinogen. Little notice, however, appears to have been taken of this observation and a review of haemorrhagic complications of pregnancy published 10 years later by Wolff and Limarzi (1946) made no mention of fibrinogen depletion or coagulation failure as possible aetiological factors.

The clinical syndrome of hypofibrinogenaemia was established by Gross and Benz (1947) in a report on the autopsy findings of three cases of amniotic fluid embolism. Fibrinogen depletion subsequently began to be widely recognised and the implication of a low plasma fibrinogen level led Moloney, Egan and Gorman (1949) to infuse fibrinogen into a patient with defective clotting associated with abruptio placentae. In the same year Dillon and Schmitz (1949) reported hypofibrinogenaemia and haemorrhage associated with fulminating eclampsia. Weiner, Reid and Roby (1950) described fibrinogen depletion in a patient with prolonged retention of a dead foetus and the same defect was subsequently reported in association with septic abortion (Conley, Ratnoff and Hartmann, 1951), secondary postpartum haemorrhage due to retained placental tissue (Nolan and Frick, 1953), and caesarean section (Leary, 1956). In more recent years it has been shown that the low level of circulating fibrinogen is not an isolated defect and reduced platelet counts together with low levels of factor V and factor VIII have been recorded in the few cases in which assays of these factors were carried out (Sharp et al, 1958; Nilsen, 1963).

The previous studies on the possible mechanisms involved in the production of a coagulation defect will not be reviewed here but will be considered in Section 3 of the thesis which deals with the coagulation and fibrinolytic systems in complications of pregnancy. Coagulation failure per se is now realised to be of less importance than the intravascular mechanisms whereby the condition is produced, as these will determine the appropriate treatment; in certain circumstances e.g. amniotic fluid embolism, the intravascular events themselves rather than the blood loss may have fatal consequences.

The Coagulation System and Pregnancy

Interest in the effect of normal pregnancy on the blood clotting mechanism was undoubtedly aroused by the acute coagulation disorders occurring in association with pregnancy and parturition. The behaviour of the coagulation system during pregnancy, however, has been the subject of conflicting reports, most probably due to the variety of clotting tests used for the assay of coagulation factors. An increase in the level of plasma fibrinogen in late pregnancy was reported by Dieckmann (1952) and this finding was subsequently confirmed in a large number of investigations (Ratnoff, Colopy and Pritchard, 1954; Fresh,

Ferguson and Lewis, 1956; Gillman, Naidoo and Hathorn, 1959; Ingram, Norris and Tanner, 1960; Talbert and Langdell, 1964). Prothrombin or factor II has been shown to be only slightly affected by pregnancy; Ratnoff et al (1954) reported an average value of 121% in late pregnancy and Pechet and Alexander (1961) found entirely normal values for prothrombin using a method which excluded reflection of factor X.

A definite increase in factor VII but no change in the level of factor V during pregnancy was reported by Alexander et al (1956) and these findings were confirmed by other investigators (Fresh et al, 1956; Kennan and Bell, 1957; Nilsen, 1963; Talbert and Langdell, 1964; Kasper et al, 1964; Todd, 1965). An increase of factor X in late pregnancy was found by Pechet and Alexander (1961), and also by Davidson and Tomlin (1963) and Nossel et al (1966).

Controversy exists regarding the effect of pregnancy on factors VIII and IX. Fresh et al (1956) and Ratnoff and Holland (1959) found that factor VIII was within normal limits during pregnancy but several more recent investigators have reported an increase not only in normal women but also in carriers of haemophilia and Von Willebrand's disease (Strauss and Diamond, 1963;

Preston, 1964; Rutherford et al, 1964; Talbert and Langdell, 1964; Nilsson and Kullander, 1967). Ratnoff and Holland (1959), Rutherford et al (1964), and Kasper et al (1964) found a marked increase of factor IX during pregnancy but other workers (Koch, 1956; Ross, 1963; Nilsson and Kullander, 1967) found no increase of factor IX and according to Todd (1965) factor IX may increase in some pregnant women but the levels reached are variable and never very high.

Fibrinolysis and Pregnancy

As with the coagulation factors, alterations in the fibrinolytic system have been reported in pregnancy. Macfarlane and Biggs (1946) mentioned that they found no evidence of plasma proteolytic activity in healthy women during pregnancy. Margulis, Luzadre and Hodgkinson (1954) and Niesert (1955) both reported a higher incidence of fibrinolytic activity in the blood during the puerperium than in the antenatal period; this was the opposite conclusion to that reached previously by Circulla and Luraschi (1953). Shea (1955) investigated fibrinolytic activity in 34 puerperal women and found activity in only two. The discrepancies in these reports were most likely due not only to different methods but also to the fact that they were

carried out at room temperature. Fearnley, Revill and Tweed (1952) demonstrated that fibrinolytic activity was quickly destroyed at room temperature but was preserved by keeping the plasma at ice temperature. Biezenski and Moore (1958) employed a low temperature method and were the first to report a gradual decrease of fibrinolysis in the blood during pregnancy - the lowest values being present in the later months of pregnancy and labour - and a rapid increase in fibrinolytic activity developing in the early postpartum period. This depression of fibrinolytic activity in pregnancy and labour was confirmed by Shaper et al (1965) and subsequently these workers (Shaper et al, 1966) reported that the return to normal fibrinolytic activity, as measured by a dilute-blood clot lysis time, took place during delivery of the baby before the cord was clamped. Brakman (1966) who recently studied fibrinolytic activity during pregnancy by assaying the activity of the euglobulin precipitate from the plasma on fibrin plates, found only a slight decrease of activity, but his assays were performed on plasma samples which had been stored at -20°C. Nilsson and Kullander (1967) measured fibrinolytic activity by determining the activity of plasma and resuspended euglobulin precipitate on unheated

fibrin plates; in the third trimester and immediately before and after delivery they found practically no fibrinolytic activity. These authors also reported that fibrinolytic activity returned to normal on the second day after confinement.

As with fibrinolytic activity the reports on the components of the fibrinolytic enzyme system during pregnancy are not in agreement. Phillips and Skrodelis (1958) found that the plasminogen level was increased during pregnancy. Shaper et al (1965) and Brakman (1966) found no difference in plasminogen levels between non-pregnant and pregnant women. Brakman and Astrup (1963) using a fibrin plate method found the capacity of the blood to inhibit urokinase, a plasminogen activator present in urine, to increase selectively and significantly during pregnancy. Using a clot lysis technique, however, Nilsson and Kullander (1967) recently reported that the inhibitors of plasminogen activation by urokinase remained unchanged during pregnancy.

The literature on the fibrinolytic enzyme system in pregnancy, therefore, presents a number of reports which are at variance with each other. This is most probably due to the use of different assay methods and the varying effect of enzymes, activators and

inhibitors of fibrinolysis in these systems. Considerable caution is hence required in the interpretation of results from the techniques at present available for the study of fibrinolysis. The recent application of immunological methods to the detection of breakdown products of fibrin or fibrinogen in the circulation has allowed a new approach to the assessment of fibrinolytic activity in vivo, as these products may reflect fibrinolysis in response to fibrin deposition occurring in normal and pathological circumstances.

The mechanisms by which the changes in coagulation and fibrinolysis are brought about in pregnancy are still obscure. A possible role of hormones, particularly oestrogen has been suggested by a number of workers (Brakman and Astrup, 1964; Brehm, 1964; Egeberg and Owren, 1964). The significance of the changes in the haemostatic mechanism during pregnancy is unknown but owing to the increase in concentration of several coagulation factors and the decreased fibrinolytic activity, pregnancy has been described as a "hypercoagulable" state (Erichson, 1965). So far, however, no causal relationship between intravascular thrombosis and increase in any known coagulation factor has as yet been established (Owren, 1965;

Johnson, 1965). The alterations of the haemostatic system in pregnancy have been interpreted as a defence mechanism against the risk of haemorrhage during parturition (Barter, 1964; Scott, 1968). The role of the coagulation system during normal parturition, however, has not been determined and the contraction of the myometrium is generally held to be the mechanism which arrests bleeding from the placental site. As discussed in the ensuing pages, a reflection of in vivo events in any particular situation is more likely to be derived from simultaneous study of the coagulation and fibrinolytic systems.

CHAPTER 3

CURRENT CONCEPTS OF THE COMPONENTS AND FUNCTIONS OF THE COAGULATION AND FIBRINOLYTIC MECHANISMS

The function of the haemostatic mechanism appears to be the maintenance of an intact and patent vascular compartment.

In physiological circumstances the coagulation and fibrinolytic mechanisms may be in a state of dynamic equilibrium, the coagulation system laying down fibrin to seal any deficiencies in the vascular endothelium and the fibrinolytic system removing the fibrin deposits after they have served their haemostatic function (Sherry et al, 1959).

Blood Clotting System

Blood coagulation can be regarded as the defence mechanism designed to preserve the blood volume in the event of injury to the vascular tree. The detailed chemistry of the complex reactions involved in blood clotting is still unknown. The 'cascade' hypothesis of coagulation proposed by Macfarlane (1964) and the similar 'waterfall' scheme of Davie and Ratnoff (1964) suggest a unified function for the clotting factors. The clotting mechanism is viewed as a series of enzyme substrate reactions,

the product of each reaction being an enzyme which reacts with the next stage of the sequence. Macfarlane (1964) has pointed out that such a sequence of reactions should act as a biochemical amplifier, the multiplicity of stages giving a corresponding increase in response to an initial stimulus. This theory also provides an explanation for the presence of the large number of clotting factors which have been identified in mammalian blood; the more stages in the amplifier, the more efficient the haemostatic response to trauma. Esnouf (1968) has recently produced evidence which modifies the original cascade described by Macfarlane and this new concept of the reactions which take place between the plasma protein factors involved in blood clotting is shown in figure 1. Two pathways have been delineated, as shown in figure 1, through which prothrombin (factor II) may be converted to thrombin as a physiological response to injury: the intrinsic mechanism which is activated by contact of the blood with surfaces other than the intact vascular endothelium and the extrinsic or tissue pathway which requires a contribution from tissue. The intrinsic pathway which depends on those factors within the plasma itself is triggered when blood or plasma comes in

contact with surfaces other than intact vascular endothelium. This activates factor XII and appears to initiate a chain of chemical reactions involving successive activation of factor IX and factor X, the factors XI, VIII and V acting as co-factors for their respective enzymes and also increasing the specificity of each stage (Esnouf, 1968). The extrinsic pathway consists of reactions which depend on contact of the blood with injured tissue which releases one or more substances known generically as tissue thromboplastin. The tissue factor reacts with factor VII to form an agent which activates factor X. Activated factor X then interacts with factor V to generate a principle capable of converting prothrombin (factor II) to thrombin. As shown in figure 1, the intrinsic and extrinsic systems converge to form the prothrombin converting principle but the nature of this principle is not yet clear. Thrombin is a highly specific proteolytic enzyme which converts the soluble protein fibrinogen into insoluble strands of fibrin, in the meshes of which are trapped cellular elements of the blood. In figure 1 the factors which have been shown to be increased in pregnancy are marked with an asterisk and such changes could represent an enhanced capacity to form fibrin particularly via the extrinsic or tissue system. The

conversion of fibrinogen to fibrin takes place in three steps (figure 2) (Scheraga and Laskowski, 1957). Thrombin splits certain peptides from fibrinogen to form individual molecules or monomers of fibrin; the fibrin monomers condense in a polymerisation step to form an insoluble fibrin network or polymer which undergoes gelation to form fibrin, the final visible clot. In the presence of fibrin stabilising factor (factor XIII) and calcium ions the clot is rendered stable and insoluble (Lorand and Jacobsen, 1958).

The Fibrinolytic System

The fibrinolytic enzyme system or plasminogen-plasmin system has a basic structure similar to that of the coagulation system and may have a physiological role complementary to that of the coagulation system in maintaining the patency of the vascular tree by promoting the removal of fibrin (Sherry et al, 1959). Though of great complexity, this system has four main components; plasminogen, plasmin, activators and inhibitors (figure 3). Plasminogen, a natural plasma β-globulin, is converted by activators to plasmin, a proteolytic enzyme which under suitable circumstances breaks down fibrin into soluble degradation products. Plasmin will also digest fibrinogen in a

manner similar to its action on fibrin and attack other plasma proteins. Plasminogen activators are found in almost all tissues of the body except the liver and the placenta and are present in especially high concentration in the uterus (Albrechtson, 1957). Using a histochemical technique, Todd (1964) has produced evidence to suggest that activity in the tissues is concentrated round blood vessels especially veins and venules. Physiological fibrinolytic activity in blood appears to be due to the presence of plasminogen activator in normal plasma (Sawyer et al, 1960).

Inhibitors of both plasmin and plasminogen activators are present in plasma and under normal physiological conditions the plasma carries a considerable excess of antiplasmin (figure 4) (Fletcher et al, 1959). Norman and Hill (1958) have shown that there are at least two antiplasmins in serum; one in the α_2 fraction (α_2-macroglobulin) reacts quickly as a competitive inhibitor of plasmin and the other in the α_1 fraction reacts more slowly but firmly with plasmin to produce an inactive complex.

Fibrinolysis In Vivo - The Sherry Hypothesis

Whenever a clot forms, plasminogen, as a result of its affinity for fibrin, is incorporated in the fibrin meshwork and it would appear that the function of plasma plasminogen is to endow any clots which may form with the means to bring about their subsequent lysis. Plasminogen appears to be present *in vivo* in a two phase system, as a soluble phase in the plasma and as a gel phase form in the thrombi and fibrinous deposits (figure 4). Plasminogen activators have a selective affinity for gel phase plasminogen (Sherry, 1966) and as a result of the physical distribution, the biochemical consequences of plasminogen activation in the two phases are entirely different. According to Sherry's hypothesis when plasminogen activator is present in plasma at physiological levels, i.e. in low concentration, it brings about lysis of fibrin by diffusing into the clot and activating plasminogen in close physical relationship to fibrin (righthand side of figure 4) and clot dissolution proceeds relatively independent of plasma inhibitors. In the plasma, on the other hand, the activation of plasminogen produces an entirely different result: under physiological circumstances plasminogen activation produces no detectable

effects on susceptible plasma proteins as the plasmin is rapidly inhibited on its formation by the antiplasmin (left-hand section of figure 4). In this manner, the action of the relatively non-specific enzyme plasmin appears to be restricted in vivo to a highly specific action, that of digestion of fibrin (Sherry et al, 1959; Fletcher et al, 1962). However, if rapid activation of soluble plasma plasminogen occurs, plasmin is produced in large amounts, the antiplasmin mechanism is temporarily overwhelmed and free plasmin appears and persists in the circulation. Fibrinogen, which is abundantly available as a subsceptible substrate can be destroyed by plasmin with the production of a severe coagulation defect.

When fibrin or fibrinogen is digested by plasmin, low molecular weight fragments are released which retain antigenic determinants of the parent fibrinogen (Nussenzweig et al, 1961). These degradation products cannot be clotted by thrombin and hence can be found in serum. Fibrin or fibrinogen degradation products are known to have a complex anticoagulant effect and have been shown "in vitro" to interfere both with normal fibrin polymerisation and the function of the platelets (Latallo, 1964;

Fletcher, 1966); for these reasons they may produce a serious haemostatic defect. On naked eye examination, clots formed in the presence of fibrin or fibrinogen degradation products are loose and friable and their abnormal structure has also been demonstrated by the electron microscope (Bang et al, 1962). The abnormality is due to the degradation products, which lack the correct structure to form a sound polymer, being incorporated with fibrin monomer.

The coagulation and fibrinolytic systems are known to react with each other at many levels e.g. both systems can be activated by activation of factor XII, platelets have antiplasmin activity; fibrinogen, factors V and VIII, and prothrombin are digested by plasmin, and fibrin degradation products have an antithrombin effect as well as inhibiting fibrin polymerisation (McNicol, 1969). Despite these observations, however, the concept of a dynamic equilibrium between clotting and lysis still remains to be firmly established.

Summary

In the past 15 years there has been a substantial increase in knowledge of the physiology of haemostasis. Pregnancy is known to be associated with an increase in the concentration of certain

coagulation factors and a decrease in the level of fibrinolytic activity but the role of the haemostatic mechanism in pregnancy and parturition is uncertain. The coagulation and fibrinolytic systems appear to be in a state of dynamic equilibrium designed to keep the vascular compartment intact and patent. In the following chapters the results of an investigation of the dual mechanism of blood coagulation and fibrinolysis in normal and abnormal pregnancy and parturition are presented and discussed.

SECTION 2

THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN NORMAL PREGNANCY AND PARTURITION

Chapter 4	Components of the fibrinolytic enzyme system in normal pregnancy, labour and puerperium
Chapter 5	Assay of fibrin degradation products and levels in normal pregnancy, labour and puerperium and after caesarean section
Chapter 6	Changes in the coagulation and fibrinolytic mechanisms during and after normal childbirth
Chapter 7	Dynamics of the coagulation and fibrinolytic mechanisms in the uterine circulation during placental separation at caesarean section
Chapter 8	The coagulation and fibrinolytic systems in the newborn

CHAPTER 4

COMPONENTS OF THE FIBRINOLYTIC ENZYME SYSTEM IN NORMAL PREGNANCY, LABOUR AND PUERPERIUM

An increase in the concentration of certain coagulation factors, particularly fibrinogen, is known to occur as pregnancy advances and the behaviour of the fibrinolytic mechanism in human pregnancy is therefore of special interest. 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.

Patients and Methods

Ten healthy women with uncomplicated pregnancies were serially studied from the first trimester to term, during labour and the puerperium. These patients were under the obstetric care of the author and full consent was given by the patients who were informed that the study related to problems with

haemorrhage during childbirth. The clinical details of these patients are shown in Table 1. 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 a ½ hour of collection of the blood which was kept at 4°C.

The following assays were performed as described in Appendix 3.

(1) Fibrinogen Assay: the biochemical method of Ratnoff and Menzie (1965) was used.

(2) Plasminogen Assay: the method of Alkjaersig et al (1959) as described by McNicol and Douglas (1964) was used. The caseinolytic assays were performed in duplicate.

(3) Euglobulin Lysis Activity: the method of Nilsson and Olow (1962) was used. Clot lysis times were measured at 37°C and the tests were carried out in duplicate. Lysis time 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 using such a plot lysis times can be expressed in arbritary units of activity assigning one unit a lysis time of 300 minutes (Sherry et al, 1959). The euglobulin lysis time is a reflection of the levels of plasminogen activator, fibrinogen and plasminogen and provided that the levels of fibrinogen and plasminogen are relatively constant, changes in the euglobulin lysis time are a measure of variations in plasminogen activator levels in the plasma.

(4) Urokinase Sensitivity Test: the method of McNicol et al (1963) was used. In this test the lysis time of a plasma clot formed in the presence of a standard amount of urokinase is measured. The lysis time reflects the overall level of inhibitors to urokinase induced fibrinolysis. Results are expressed in units based on a double logarithmic plot with a lysis time of 10 minutes arbitrarily assigned one unit of activity. The lower the number of units the lower the sensitivity to urokinase induced lysis and, correspondingly, the higher the degree of fibrinolytic inhibition.

(5) Thrombin Clotting Time: the method described by Fletcher

et al (1959) was used and the tests were performed in duplicate. The clotting time is prolonged by degradation products of fibrin or fibrinogen.

(6) The Platelet Count: the method of Dacie (1963) was used and duplicate counts were performed on venous blood.

Results

(1) Fibrinogen The previously documented increase of plasma fibrinogen during pregnancy was confirmed as illustrated in figure 5 and Table 2. The mean fibrinogen level was slightly increased in early pregnancy at 345mg/100ml compared with a mean level of 268mg/100ml found six weeks after delivery (Table 8). The fibrinogen level gradually increased with the duration of pregnancy to a mean value of 443mg/100ml 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.

(2) Plasminogen The levels of plasminogen followed serially through pregnancy are shown in figure 5 and Table 3. Elevated

levels of plasminogen were found as early as the fourth month of pregnancy and 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 elevated but by the sixth postnatal week the levels were in the normal non-pregnant range.

(3) Euglobulin Lysis Activity A marked decrease of fibrinolytic activity during pregnancy was found, the euglobulin lysis time increasing steeply after the first trimester (figure 6). 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 ($R = -0.839$, $p<0.001$) as shown in figure 7 and Table 4. The increase in fibrinolytic activity in the first week of the puerperium from the values found in the third trimester and in labour was highly significant ($t = 5.56$, $p<0.001$).

(4) Urokinase Sensitivity Test In this study normal pregnancy did not appear to be associated with any significant change in the level of inhibitors of plasminogen activation by urokinase (Table 5).

(5) Thrombin Time No significant alteration of thrombin time took place during pregnancy, but a significant increase was found in labour from the values present at 36-42 weeks of pregnancy (Table 6).

(6) 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. As shown in figure 8 and Table 7. a significant rise of the platelet count was found in the first week of the puerperium as compared with the level found in labour ($t = 2.4$, $p<0.02$).

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 at an unstated time in pregnancy 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 more recent findings of Nilsson and Kullander (1967). The increase of plasminogen as shown in figure 5 appears to occur pari passu with that of fibrinogen and the rise above normal levels in the third 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. The data presented therefore suggest that the decreased fibrinolytic activity observed during pregnancy represents a decrease in the level of circulating plasminogen activator, as although the plasma fibrinogen level is elevated, 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 this study normal 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.

No significant alteration in the platelet count was found 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 recently 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 is that the changes during pregnancy may be a physiological development to ensure the integrity of both the maternal and foetal circulations but such alterations may also establish a vulnerable state for intravascular fibrin deposition.

The hypothesis of transient coagulation in the blood which can obstruct the micro-circulation 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 than in non-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 intra-vascular coagulation and haemorrhage.

CHAPTER 5

THE ASSAY OF FIBRIN DEGRADATION PRODUCTS AND THE LEVELS DURING NORMAL PREGNANCY, LABOUR AND THE PUERPERIUM, AND AFTER CAESAREAN SECTION

The appreciation of the importance of the fibrinolytic mechanism in recent years has stimulated the evolution of new methods which enable more accurate assessment of the level of activity of the fibrinolytic enzyme system. When the proteolytic enzyme plasmin breaks down fibrin or fibrinogen, degradation products of these proteins appear in the circulation. Some of the fragments released are incapable of undergoing further digestion by plasmin but they retain antigenic determinants of the parent fibrinogen (Nussenzweig et al, 1961; Alkjaersig et al, 1962); certain degradation products cannot be clotted by thrombin and hence can be found in serum. Degradation products of fibrinogen have been shown to interfere with the thrombin-fibrinogen reaction (Latallo et al, 1964), the polymerisation of fibrin (Fletcher, 1966), and the aggregation and adhesion of platelets (Kowalski et al, 1964; Wilson et al, 1968); for these reasons

fibrin/fibrinogen degradation products can produce a serious haemostatic defect.

Immunological methods have recently been applied to the assay of fibrinogen/fibrin degradation products and of these techniques the haemagglutination inhibition assay with sensitized red cells as developed by Merskey et al (1966) is one of the most sensitive. The technique was evolved from the principles used in the quantitative assay of chorionic gonadotrophin and growth hormone (Wide, 1962; Read et al, 1962). The basic reagents in the assay are antifibrinogen antiserum, fibrinogen coated red cells and the unknown serum sample.

The unknown serum sample is first titrated with a known amount of the antiserum which is then bound proportionately to the concentration of antigenic fibrinogen material in the sample. The unbound fraction of the antiserum is next quantitated by adding fibrinogen coated red cells and this allows the level of antigenic fibrinogen material in the unknown sample to be calculated.

The accurate assay of circulating fibrin/fibrinogen degradation products is of considerable importance in the

investigation of physiological and pathological mechanisms in coagulation and fibrinolysis as they represent good evidence that fibrinolysis is taking place. The following investigation was designed to establish the normal range of fibrinogen/fibrin degradation products in the serum during normal pregnancy, labour and the puerperium and after caesarean section.

Patients and Methods

The levels of fibrin/fibrinogen degradation products were studied in 250 healthy women during uncomplicated pregnancy, labour and the puerperium, and in 12 patients following delivery by lower uterine segment caesarean section. The blood samples for this study were obtained when routine blood tests were being taken during pregnancy, labour and the puerperium.

Preparation of samples

The blood samples (5ml) from an antecubital vein were added immediately to plastic tubes containing glass beads and 1mg of the fibrinolytic inhibitor tranexamic acid to inhibit fibrinolysis occurring in vitro. Specimens were incubated at 37°C for two hours to ensure complete fibrinogen-fibrin conversion and the serum was then separated and stored in

plastic tubes at -20°C till assayed. Each tube had a code number and when the assays were read the clinical details were unknown.

Method of assay

The assay was performed according to the method of Merskey et al (1966) with minor modifications. All the serum samples were adsorbed at 4°C for two hours with 1/5 volume of packed fresh sheep red cells and then assayed immediately for fibrin/fibrinogen degradation products. The microtitre system (Cooke Engineering Co., California) was employed and the sheep cells sensitised with fibrinogen were from the same batch throughout the whole investigation. The preparation of sheep red cells and sensitisation procedure together with details of the assay method are described in Appendix 3. Rabbit anti-human fibrinogen serum (Hoechst Pharmaceuticals Ltd.) was used at a dilution of 1/4,000 and the citrate buffer for the doubling dilutions contained tranexamic acid in a concentration of 20mg/100ml. Three concentrations of a fibrinogen standard, either a solution of purified human fibrinogen (Kabi Pharmaceuticals Ltd.) or a standard pooled plasma solution were included in each batch of assays. The

clottable protein in the standard solutions was assayed by the method of Ratnoff and Menzie (1965). The precision of the assay for fibrin/fibrinogen degradation products in serum was increased by using intermediate dilutions and the method was reproducible down to a level of 0.6-1μg/ml.

In patients where a prolonged thrombin clotting time (McNicol and Douglas, 1964) was present the test serum was incubated for 2 hours at $37^{o}C$ with approximately 1/10 of its volume of thrombin (100units/ml) to remove any residual thrombin clottable fibrinogen. In the test samples where elevated levels were demonstrated the assay was repeated after further incubation with thrombin so that as far as possible only immunological reactive products were estimated which were not clottable by thrombin.

Results

The results of the assay for fibrin/fibrinogen degradation products in the serum during pregnancy, labour and the puerperium in the 250 patients are shown in figure 9 and Table 9. The mean level between the 18th and 24th week of pregnancy was 1.25 ± 0.53μg/ml and no significant alteration of the level took place until the first stage of labour when the mean level rose to

3.45 $\pm$ 2.8µg/ml, a highly significant rise ($t = 4.555$, $p<0.001$). A further increase in the mean level was found during the first week of the puerperium; by the 6th week after delivery the mean serum level had returned to 1.86 $\pm$ 1.7µg/ml.

In the 12 patients studied after caesarean operations the levels of fibrin/fibrinogen degradation products 2-4 hours after operation were in the range 1.6-20.0µg/ml (mean 5.83µg/ml). Samples from the same 12 patients taken 3-8 days after operation were in the range 1.5-108µg/ml (mean 21.0µg/ml), a significant rise (figure 10 and Table 10). The levels were thus substantially higher after caesarean operations than after normal delivery.

Discussion

The investigation of the fibrinolytic enzyme system in normal pregnancy (Chapter 4) showed that the rise of plasma fibrinogen during pregnancy was accompanied by a proportionate elevation of plasma plasminogen, and the level of circulating plasminogen activator was markedly diminished in late pregnancy and throughout labour. The immunological assay employed in this study does not distinguish between the degradation products of fibrin and those of fibrinogen. It is of interest that no significant

alteration of the level of degradation products could be shown during pregnancy despite the increase of plasma fibrinogen. A recent study by Woodfield et al (1968) reported progressively higher levels of degradation products in the second and third trimester, but in the investigation reported in this chapter no significant increase of the level was found until the onset of labour.

The sharp increase in the levels of degradation products during labour is reliable evidence that active fibrinolysis is taking place. Increased amounts of fibrin/fibrinogen degradation products could explain the significant increase in the thrombin clotting time found during labour, reported in Chapter 4 (Table 6), as these products are known to interfere with the thrombin-fibrinogen reaction (Latallo et al, 1964). The rise of degradation products takes place in the presence of a markedly diminished fibrinolytic activity in the circulation, a consistent finding in labour, indicating that active fibrinolysis may be present in vivo which is not reflected in the level of plasminogen activator found for example in venous blood taken from the arm. Lysis of circulating fibrinogen is not likely to take place in the presence of diminished systemic fibrinolytic activity. This suggests that

the degradation products are most probably a consequence of lysis of fibrin in localised areas of the vascular compartment. Fibrin plaques and numerous intervillous thrombi are found on the maternal side of the majority of normal placentae (Fox, 1963; Devi et al, 1968). The uterus is a potent source of plasminogen activator (Albrechtson, 1957; Mackay, 1967) and uterine action during labour is accompanied by transient myometrial ischaemia. Circumstances therefore may exist for the local release of activator and this may then be absorbed on placental fibrin with consequent local fibrinolytic activity in the placental circulation. An enhanced fibrinolytic activity in the uterine circulation during labour could be a natural defence mechanism to maintain the maternal blood supply to the placenta by ensuring the patency of the placental bed during labour.

The elevated levels of degradation products in the puerperium after normal delivery are possibly related to the removal of fibrin laid down to secure haemostasis in the uterus after separation of the placenta. The higher levels found after delivery by caesarean section may possibly reflect the more extensive tissue damage associated with operative delivery.

These findings suggest that in normal pregnancy and parturition

the level of fibrin degradation products in the circulation may be the end result of a complex dynamic system which adapts to local requirements in the uterine circulation during normal labour and after delivery.

CHAPTER 6.

CHANGES IN THE COAGULATION AND FIBRINOLYTIC MECHANISMS DURING AND AFTER NORMAL CHILDBIRTH

Parturition presents a serious challenge to the integrity of the vascular compartment, but it has been generally held (Taylor, 1966; Donald, 1969) that contraction of the myometrium - the "living ligatures" of the uterus - is the mechanism which, in the main, controls blood loss at delivery. In other situations where there is injury to the vascular tree an efficient blood coagulation system plays a vital role in achieving effective haemostasis and preserving the blood volume. In this chapter is described a detailed sequential study of the coagulation and fibrinolytic systems which was designed to determine the effect of the actual process of normal childbirth and placental separation on the haemostatic mechanism.

Patients and Methods

Fifteen healthy patients with uncomplicated full term pregnancies were studied and full and informed consent was given by each patient for specimens of blood to be taken during and following delivery. The timing of the blood samples was as follows : (1) Late in the second stage of labour when the baby's

head was distending the perineum. (2) Immediately after delivery of the baby while the placenta was separating in utero i.e. third stage of labour. (3) Within one minute of expulsion of the placenta. (4) Fifteen minutes after placental delivery. (5) One hour after placental delivery. (6) Twenty-four hours after delivery. (7) Three to five days after delivery.

The blood samples (16ml) were taken into plastic syringes from the arm vein with the minimum of venous occlusion: 9ml of blood was mixed with 1ml of 3.8% sodium citrate in a plastic tube for coagulation and fibrinolytic tests, 5ml was added to a plastic tube containing glass beads and 1mg of tranexamic acid for assay of fibrin/fibrinogen degradation products, and 2ml to a plastic tube containing EDTA for platelet counting. The coagulation and fibrinolytic assays were invariably carried out within a half-hour of collection of the blood which was kept at 4°C.

The following assays were performed as described in Appendix 3.

(a) Coagulation System Assays

Recalcification time in plastic tubes.

Kaolin-cephalin clotting time (Proctor and Rapaport, 1961).

Partial thromboplastin time (Langdell et al, 1953).

Factor II (Prothrombin) (Owren and Aas, 1951).

Factor V (Shanberge et al, 1967).

Factors VIII and IX (Breckenridge and Ratnoff, 1962).

Factor X (Denson, 1961).

One stage prothrombin time (Douglas, 1962).

Thrombin clotting time (McNicol and Douglas, 1964).

Platelet count (Dacie, 1963).

(b) Fibrinolytic Enzyme System Assays

Fibrinogen (Ratnoff and Menzie, 1965).

Plasminogen (Alkjaersig et al, 1959; McNicol and Douglas, 1964).

Euglobulin lysis time (Nilsson and Olow, 1962).

Urokinase sensitivity test (McNicol et al, 1963).

Fibrin/fibrinogen degradation products (Merskey et al, 1966; Bonnar et al, 1969c).

Statistical Analysis. As the data comprised measurements on a single group at different times, Wilcoxon's Signed Ranks Test was used to determine the statistical significance of any changes in the coagulation and fibrinolytic assays. The principles of this test are described in Appendix 3.

Results

(a) Coagulation Systems Assays

Tables 11 to 21 contain the results of the coagulation tests in the 15 patients during and after delivery. Figure 11 shows the significant shortening of the plasma recalcification time, kaolin-cephalin clotting time and partial thromboplastin time which occurred during the third stage of labour and within one minute of placental delivery. The recalcification time decreased sharply during the third stage of labour and immediately after placental delivery ($R = 0$, $p<0.002$); thereafter the recalcification time gradually increased and the pre-delivery range of values were again found on the first day of the puerperium. The kaolin-cephalin clotting time showed a significant but less dramatic shortening during the third stage and immediately after placental delivery ($R = 6.5$, $p<0.05$). The plasma thromboplastin time shortened during the third stage of labour and immediately following placental delivery ($R = 11.0$, $p<0.01$) but within 15 minutes the pre-delivery values were restored.

Figure 12 shows the changes in the coagulation factors which occurred before and after delivery. A sharp increase in

factor VIII activity occurred during delivery, the mean factor VIII level rising from 212% in the second stage of labour to 251% immediately after placental delivery ($R = 2.5$, $p<0.01$). The pre-delivery values of factor VIII activity were restored one hour after delivery and the mean level remained around 200% in the immediate puerperium. The mean level of factor V increased immediately following placental delivery and on the first day of the puerperium the levels of factor II (prothrombin) and factor V were significantly lower than during the first hour after placental delivery. A normal level of factor IX activity was recorded in the second stage of labour and after placental delivery but a slight increase of the mean level was recorded in the early puerperium. Factor X activity was increased during the second stage of labour with a mean value of 166% and the mean level decreased to 112% between the 3rd and 5th day of the puerperium.

As shown in figure 13 no significant change occurred in the prothrombin time and thrombin clotting time but a highly significant increase of the platelet count was evident following delivery, the mean platelet count increasing from 221,000 per

cmm during delivery to 304,000 per cmm between the 3rd and 5th day of the puerperium (R = 0, $p<0.002$).

(b) <u>Fibrinolytic Enzyme System Assays</u>

Tables 22 to 26 contain the results of the fibrinolytic studies during and after delivery. Figure 14 shows the significant decrease of plasma fibrinogen which occurred during the third stage of labour and in the first 15 minutes following delivery of the placenta (R = 0, $p<0.002$). The fibrinogen level then gradually increased and was at a higher level three to five days after delivery than during the second stage of labour. The plasminogen level showed a slight but significant decrease during the third stage of labour and immediately after placental delivery (R = 8.5, $p<0.01$) but no increase of plasminogen was evident in the early puerperium. The decrease of fibrinogen and plasminogen during the third stage of labour and immediately following placental delivery showed a significant positive correlation ($r = +0.327$, $p<0.05$) (figure 15). A marked inhibition of euglobulin lysis activity was present in the second and third stage of labour and for the first 15 minutes after delivery but within one hour euglobulin lysis activity was in the normal

non-pregnant range and fibrinolytic activity continued in the normal range in the immediate puerperium. The change in the euglobulin lysis activity between 15 minutes and one hour after placental delivery was highly significant ($R = 0$, $p \leq 0.002$). No change in the level of inhibitors to urokinase induced lysis was detected until the first day after delivery when a slight but significant increase in the level of inhibitor was present ($R = 6.5$, $p \leq 0.002$). During the second stage of labour the levels of fibrin/fibrinogen degradation products in the serum were increased but showed a wide range of values ($3.8 \pm 2.9 \mu g/ml$) and one hour following the delivery a further increase took place, the level increasing to $5.8 \pm 4.7 \mu g/ml$. The levels remained elevated on the first day of the puerperium and decreased slightly between the 3rd and 5th day of the puerperium.

Discussion

Haemorrhage during childbirth is often associated with uterine atony or soft tissue laceration but in these circumstances bleeding is usually readily controlled and is rarely fatal. Although the precise mechanism of haemostasis during normal parturition is still uncertain it has been shown in recent years

that fatal obstetric haemorrhage is frequently accompanied by defective blood clotting.

The concentration of plasma fibrinogen and of factors VII and X is increased in late pregnancy (Ratnoff et al, 1954; Pechet and Alexander, 1961; Talbert and Langdell, 1964) and recent studies have shown that factor VIII activity is also increased (Nossel et al, 1966; Nilsson and Kullander, 1967). Fibrinolytic activity is known to be decreased in late pregnancy and labour and to return to normal in the early puerperium (Biezenski and Moore, 1958; Shaper et al, 1965; Bonnar et al, 1969). The findings reported in this chapter concerning the coagulation and fibrinolytic systems in the second stage of labour are largely in agreement with previous reports on these systems in late pregnancy.

In a previous study of the blood clotting mechanism during normal parturition, Ratnoff et al (1954) were unable to demonstrate any consistent changes in the clotting tests; the tests performed were the whole blood clotting time, thrombin time, plasma fibrinogen, prothrombin assay and platelet count and only a few of these tests were performed on any one patient. The sequential

study presented here has shown that striking changes do take place in the coagulation and fibrinolytic systems during the actual process of parturition.

In the coagulation system assays the shortening of the recalcification time, kaolin cephalin clotting time and partial thromboplastin time and the sharp increase in factors VIII and V activity in the peripheral blood during and immediately following placental separation suggests that activation of the clotting mechanism takes place during parturition. Further, the significant decrease of fibrinogen levels during and after placental separation would be in keeping with local deposition of fibrin. The decreased levels of factors II and V on the day following delivery may reflect utilisation of these factors or their digestion by plasmin.

The changes in the fibrinolytic components during and immediately following placental delivery are consistent with fibrinolysis occurring as a response to local fibrin deposition; the plasma plasminogen level decreased during the third stage of labour and after placental delivery, and the level of fibrin/fibrinogen degradation products in the serum increased one hour after childbirth and remained elevated in the early puerperium.

Fibrinolytic activity as measured by the euglobulin clot lysis assay remained markedly reduced during the second and third stage of labour and the sudden change from reduced to normal activity took place between 15 minutes and one hour after delivery. Biezenski and Moore (1958) using a semi-quantitative plasma clot lysis method found that lytic activity returned to normal within three hours of delivery. Shaper et al (1966) reported that in African women the return to normal fibrinolytic activity occurred as the child was delivered and before the cord was clamped. The mechanism by which fibrinolytic activity abruptly returns to normal within one hour of delivery is unknown. Kawano et al (1968) showed that placental extracts contain high levels of inhibitors of fibrinolysis and he suggested that the placenta itself is responsible for the fibrinolytic inhibition which persists until after the placenta is delivered.

The concurrent findings in the blood coagulation and fibrinolytic systems seem therefore to indicate that during parturition the haemostatic mechanism has an important complementary function to the unique process of myometrial contraction,which by extravascular compression diminishes the blood flow to the placental site. Life threatening postpartum

haemorrhage is a common complication in women severely affected by Von Willebrand's disease, especially when the factor VIII level is low and does not increase during pregnancy (Biggs, 1966; Walker and Dormandy, 1968), suggesting that the clotting mechanism has a vital role in preventing blood loss during parturition. Likewise in abruptio placentae where a marked depletion of clotting factors may develop, serious and sometimes fatal haemorrhage from the uterus is not uncommon. The increased levels of clotting factors during normal pregnancy may represent therefore a physiological development to provide for the rapid and effective haemostasis which is required during parturition.

It is of interest that in the early puerperium a secondary increase takes place in the level of fibrinogen, factor VIII activity remains well above normal non-pregnant levels, and the platelet count shows a steep rise. During the puerperium the incidence of thrombo-embolic complications is three to four times greater than during pregnancy (Husni et al, 1967). The rapid rise in circulating platelets and the increased levels of fibrinogen and factor VIII together with the limited activity of the mother which follows childbirth, are possible explanations

of the predispostion to thrombosis during the puerperium. The abrupt return of normal fibrinolytic activity after delivery may be a protective mechanism to combat this hazard.

CHAPTER 7

DYNAMICS OF THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN THE UTERINE CIRCULATION DURING PLACENTAL SEPARATION AT CAESAREAN SECTION

As changes in the blood clotting and fibrinolytic systems had been detected in peripheral blood during normal parturition, an attempt was made to find out if these changes were a reflection of events in the uterine circulation. The behaviour of the coagulation and fibrinolytic systems was therefore investigated simultaneously in the uterine circulation and peripheral blood during delivery by caesarean section.

Patients and Methods

Twelve patients with haemoglobin levels over 12g/100ml were studied during and after delivery by elective caesarean section. The patients were under the obstetric care of the author and full consent was given by each patient for the blood samples to be taken as part of an investigation into the causes of haemorrhage during childbirth. After incision of the abdominal wall and exposure of the uterus, blood samples were taken simultaneously from a large vein on the surface of the uterus in the region of the placental site and from an

arm vein. A lower uterine segment caesarean operation was then performed. Immediately after delivery of the baby, blood samples were taken simultaneously from a vein at the uterine fundus draining the placental bed and from an arm vein, these specimens being taken while the placenta was separating spontaneously 'in utero'. The placenta was then delivered by cord traction and at intervals of five and fifteen minutes after delivery of the placenta, blood samples were taken from veins draining the placental site and simultaneously from arm veins. After closure of the abdominal wall, approximately 25 minutes after placental delivery, a blood sample was taken from an arm vein. In the post-operative period peripheral blood samples were taken at 24-48 hours, 3-5 days, 6-9 days, and 10-14 days after operation. In several instances blood samples were not obtained at all the times specified due to the absence of an adequate number of suitable veins.

The blood samples (16-18ml) were taken by clean venepuncture into plastic syringes: 2ml was used for measuring the whole blood clotting time, 9ml was mixed with

1ml of 3.8 per cent sodium citrate in a plastic tube for coagulation and fibrinolytic tests, 3-5ml was added to a plastic tube containing glass beads and 1mg of tranexamic acid for assay of fibrin/fibrinogen degradation products, and 1-2ml was added to a plastic tube containing EDTA for platelet counting. When blood samples were obtained at all the times specified the total quantity of blood removed amounted to 144ml during the operation and 80ml during the two weeks after operation.

The whole blood clotting time was measured in the operating theatre in a portable water bath at 37°C and the coagulation and fibrinolytic assays were performed within a half hour of collection of the blood which was kept at 4°C.

The following assays were performed as described in Appendix 3.

(a) Coagulation System Assays

Whole blood clotting time in plastic tubes

Recalcification time in plastic tubes

Kaolin-cephalin clotting time

Partial thromboplastin time

One stage prothrombin time

Thrombin clotting time

Factors V, VIII and IX

Platelet count

(b) Fibrinolytic Enzyme System Assays

Fibrinogen

Plasminogen

Euglobulin lysis activity

Urokinase sensitivity test

Fibrin/fibrinogen degradation products

Statistical Analysis

As the measurements were from a single group at different stages, Wilcoxon's Signed Ranks Test was used to determine the statistical significance of any changes in the coagulation and fibrinolytic tests. The principles of this test are outlined in Appendix 3.

Results

(a) Coagulation System Assays

Tables 27-35 contain the results of the coagulation tests in the uterine vein blood and the peripheral blood taken simultaneously before delivery and during and after placental separation in the 12 patients, and the results in the peripheral

blood in the two weeks following operation.

Figure 16 shows the striking shortening of the whole blood clotting time and recalcification time in plastic tubes (Tables 27 and 28) which was found in the uterine blood while the placenta was separating in utero. In two patients the blood sample taken from the uterine vein during placental separation clotted within seconds in the plastic syringe. The whole blood clotting time and recalcification time in the uterine vein blood during placental separation were significantly shorter than the times recorded in the peripheral blood taken simultaneously ($R = 0$; $p<0.01$). While the placenta was separating the platelet count in uterine blood showed a slight decrease but no change was detected in peripheral blood during or immediately after placental separation. In the post-operative period a marked rise in the platelet count was found, the mean level steadily increasing from 229,000 per cmm at 2-4 hours after operation to 478,000 per cmm at 10-14 days after operation (Table 29 and figure 16).

Figure 17 shows that during placental separation the shortening of the kaolin cephalin clotting time (Table 30), partial thromboplastin time (Table 31), and the one stage

prothrombin time (Table 32) was more marked in the uterine vein blood than in peripheral blood. No significant change was found in the thrombin clotting time in uterine or peripheral blood during or after placental separation (Table 33).

Figure 18 shows the striking increase in factor VIII activity found in both uterine vein blood and peripheral blood during and after placental separation; the mean factor VIII activity in uterine blood rose from 141% before delivery to 336% during placental separation, and the simultaneous samples of peripheral blood showed an increase from 148% before delivery to 228% during placental separation. Factor VIII activity as shown in figure 18 and Table 34 remained elevated in both uterine blood and peripheral blood following delivery of the placenta but 2-4 hours after operation the mean factor VIII activity in peripheral blood had decreased to 155%. In the two weeks following delivery by caesarean section the mean factor VIII activity was approximately 200% with a fall to 143% at 10-14 days after operation. No change was found in the level of factor IX or factor V in uterine or peripheral blood with the exception of a slight increase of factor IX activity 24-48 hours following delivery (Tables 35 and 36).

As shown in figure 19 and 20, the shortening of the kaolin cephalin clotting time and partial thromboplastin time which was found in uterine blood during and immediately after placental separation showed a significant negative correlation ($p<0.01$) with the increase of factor VIII activity in uterine blood at this time.

(b) Fibrinolytic Enzyme System Assays

Tables 37-41 contain the results of the fibrinolytic studies. The only change found in the plasma fibrinogen level was a decrease in the peripheral blood following the operation, the mean plasma level falling from approximately 400mg during the operation to 346mg/100ml 2-4 hours after the operation (figure 21 and Table 37). During the two weeks after delivery by caesarean section the plasma fibrinogen level steadily increased and the highest level was present at 10-14 days after operation when the mean level had risen to 536mg/100ml. As shown in figure 21 the mean plasma plasminogen level decreased slightly in both uterine blood and peripheral blood, following placental separation and during the two weeks after operation the mean plasminogen level remained virtually unchanged (Table 38).

Figure 22 shows the dramatic increase in the euglobulin lysis activity which was found in uterine blood during and immediately after placental separation, the level of activity being considerably higher than that recorded in the peripheral blood taken at the same time ($R = 3$, $p<0.05$). A marked inhibition of euglobulin lysis activity was present in the peripheral blood during and immediately after separation of the placenta but at 2-4 hours after operation euglobulin lysis activity was in the normal non-pregnant range and remained so in the two weeks after operation (figure 22 and Table 39). The level of fibrin/fibrinogen degradation products increased slightly in uterine blood during placental separation; in the peripheral blood the mean level increased from 3.53μg/ml at the end of the operation to 6.33μg/ml 2-4 hours later and during the two weeks after operation a wide range of values was found with peak levels at 6-9 days after operation when the mean value was 13.43μg/ml (figure 22 and Table 40). No change in the level of inhibitors to urokinase induced lysis was detected in the uterine blood during or after placental separation and in the peripheral blood the only change was a transient increase in the level of urokinase inhibition occurring immediately following placental separation (figure 22 and Table 41).

DISCUSSION

In normal circumstances blood clotting takes place at sites of vascular injury. The evidence presented here indicates that in the uterine circulation a distinct activation of the coagulation and fibrinolytic systems takes place during childbirth at the time of placental separation. The wide difference in activity between uterine blood and peripheral blood taken simultaneously indicates that in vivo a pronounced local activation of the clotting mechanism may take place which produces only minimal changes in blood taken remote from the area involved. This was particularly evident in the shortening of the whole blood clotting time in plastic tubes during placental separation when six of the twelve patients had clotting times of three minutes or less in the uterine blood but only a slight shortening of the time took place in the peripheral blood. A similar but less pronounced pattern of results was also found in the recalcification time, kaolin cephalin clotting time, partial thromboplastin time, prothrombin time, and the platelet count.

A definite increase of factor VIII activity was found in uterine blood and to a lesser extent in peripheral blood during and immediately after placental separation but the factor IX level

remained virtually unchanged. The assay procedure for factor IX was identical to that of factor VIII except that the substrate plasma employed was deficient in factor IX; the sharp increase of factor VIII activity cannot therefore be attributed to the presence of thromboplastin or some other clotting accelerator which could influence the assay procedure. At the present time very little is known of the detailed chemistry of the pathways of the blood clotting mechanism. Recent studies have indicated, however, that when the coagulation sequence is activated the first traces of thrombin formed appear to act first on factor VIII producing a more reactive molecule which subsequently accelerates the intermediate phases of blood coagulation (Ozge-Anwar et al, 1965; Rapaport et al, 1965; Davie et al, 1969). The shortening of the clotting time, kaolin cephalin time and partial thromboplastin time is related to the sudden increase of factor VIII activity in the uterine circulation during placental separation and probably reflects thrombin activity at the placental site due to the local release of tissue thromboplastin.

Brown and Stalker (1969) showed in the hamster that thromboplastin material can enter the maternal and foetal

circulation during placental separation. The investigation of normal childbirth described in Chapter 6 showed that transient shortening of clotting tests and an increase of factor VIII activity take place in peripheral blood during placental separation. The findings reported in this chapter suggest that these changes are directly due to activation of the clotting mechanism in the uterine circulation producing to a lesser extent a coagulant effect in peripheral blood.

The potency of the mechanisms for keeping the clotting of blood a local phenomenon and clearing the activated clotting factors from the circulation is shown by the fact that the pronounced changes in the uterine vein were transitory and had almost disappeared by the time the blood reached the peripheral circulation. This is probably why such a pronounced coagulant effect has not been noted previously during parturition. When studying changes in the haemostatic mechanisms caused by alterations in a single organ it seems important, therefore, to examine the blood directly leaving the organ, as the changes may not be detectable in the peripheral circulation. The plasma contains potent inhibitors of such factors as thrombin (Seegers and Marciniak, 1962), and activated factor X (Biggs et al, 1970),

and activated clotting factors are apparently removed by the liver and the reticulo-endothelial system (Spaet, 1962).

Fibrinolytic activity was reduced in both uterine blood and peripheral before delivery but during placental separation the level of activity in the uterine blood was considerably increased compared with that of the peripheral blood - findings possibly due to plasminogen activator entering the circulation at the placental site. After delivery by caesarean section fibrinolytic activity in the peripheral blood followed a similar pattern to that found after normal vaginal delivery (Chapter 6; Bonnar et al, 1970), the euglobulin lysis activity being in the normal range at 2-4 hours after operation.

The levels of fibrinogen, plasminogen and factor VIII were decreased two to four hours after operation and at the same time levels of fibrin/fibrinogen degradation products in the peripheral blood were increased. In the haemorrhagic complications of pregnancy, particularly abruptio placentae, much more striking changes, but in the same direction, have been found (Verstraete et al, 1965; Bonnar et al, 1969 b). Clearly, effective haemostasis in the uterus during placental separation places heavy demands on

the coagulation factors, and the high levels found in normal pregnancy will provide a reserve of haemostatic components to meet this challenge.

The changes in the coagulation and fibrinolytic systems in the two weeks after caesarean section are of special interest as these may be aetiological factors involved in thrombo-embolic complications which occur during this period. The raised factor VIII level and the progressive increase in the number of circulating platelets and fibrinogen concentration over the two weeks after operation may be of particular importance in predisposition to thrombosis; the increased production of these factors may have been stimulated by their utilization during placental separation. Emmons and Mitchell (1965) reported that the maximal increase in both the total platelet count and aggregation were present around the 10th post-operative day. The increase in the levels of fibrin/fibrinogen degradation products which was maximal at 6-9 days after operation could reflect lysis of intravascular fibrin at this time and suggests that the stimulus to coagulation continues for relatively long periods. If active intermediates of clotting

continue to circulate they could contribute to the incidence of thrombosis in the puerperium. The changes of the haemostatic mechanism following caesarean section would appear to favour intravascular clotting and such changes together with the limited physical activity which often follows operative delivery may explain the relatively high incidence of thrombo-embolic complications after caesarean section.

It has long been suspected that activation of the coagulation mechanism may occur in a small part of the circulation and yet produce little evidence of its existence in conventional tests applied to blood collected from an antecubital vein. The observations reported in this chapter confirm the interpretation of the changes in peripheral blood during normal parturition and delivery by caesarean section, as evidence for the stimulation of coagulation and fibrinolysis locally in the uterus. This study also underlines the significance to be attached to raised levels of factor VIII in one-stage assays on peripheral blood as evidence for the existence of the local stimulation of coagulation somewhere in the body (Penick et al, 1965). These observations do not diminish the importance of

myometrial contraction in the control of bleeding after childbirth but they indicate that the coagulation system also plays a vital role in the process.

CHAPTER 8.

THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN THE NEWBORN

Haemorrhage is still an important cause of perinatal morbidity and mortality particularly in the premature infant. The newborn infant is known to have a deficiency of clotting factors but to the author's knowledge there has been no simultaneous and comprehensive study of the coagulation and fibrinolytic systems in the mother and cord blood of the infant at the time of delivery. The findings presented in Chapters 6 and 7 indicate that in the maternal blood a pronounced activation of the coagulation and fibrinolytic systems takes place in the uterine circulation during placental separation. As the process of placental separation commences during the delivery of the infant it may also have an effect on the haemostatic system of the infant as well as the mother. This chapter presents the results of a detailed investigation of the blood coagulation and fibrinolytic mechanisms in the full term infant and the mother immediately following delivery.

Patients and Methods :

Ten healthy women,at term with uncomplicated pregnancies,and their newborn infants were studied and full consent was given by the mother for the specimens of blood to be taken. The blood samples were taken immediately following delivery of the baby while the umbilical cord was pulsating and prior to clamping of the cord. The baby's blood sample (15 ml.) was taken into a plastic syringe by clean venepuncture of the umbilical vein. Clamping of the cord was delayed until pulsation had ceased to allow an extra quantity of blood to pass to the infant which would more than compensate for the venous blood sample. The mother's blood sample (15 ml.) was taken from an arm vein simultaneously with that from the baby. The 15 ml. blood sample was subdivided immediately as follows : 9 ml. was mixed with 1 ml. of 3.8% sodium citrate in a plastic tube at 4°C. for coagulation and fibrinolytic tests, 4 ml. was added to a plastic tube containing glass beads and 1 mg. of transexamic acid for assay of fibrin/fibrinogen degradation products, and 2 ml. was added to a plastic tube containing edetic acid for platelet counting.

The coagulation and fibrinolytic assays were performed within a half-hour of collection of the blood which was kept at 4°C. The following assays were performed by methods described in Appendix 3.

Coagulation System Assays :- Recalcification time in plastic tubes, kaolin-cephalin clotting time, partial thromboplastin time, thrombin clotting time, prothrombin time, coagulation factors II, V, VIII, IX and X, and platelet count. Factor VII was assayed by a one-stage method using plasma with a congenital deficiency of factor VII (Biggs and Macfarlane, 1962).

Fibrinolytic Enzyme System Assays :- Fibrinogen, plasminogen, euglobulin lysis activity, urokinase sensitivity test, and serum fibrin/fibrinogen degradation products.

Statistical Analysis :- Wilcoxon's Signed Ranks Test was used in the statistical analysis of the paired measurements for the mother and the baby.

RESULTS

The results of the coagulation system assays in the newborn and in the mother immediately after birth are listed in

Tables 42, 43A and 43B, and the mean levels are compared in Figures 23 and 24. Factor VII assays were performed in six of the ten babies due to insufficient test plasma in the others (Table 43B). With the exception of the recalcification time in plastic a highly significant difference was found between the clotting tests in the newborn and the mother. The most obvious difference was a prolongation in the newborn of the Kaolin-cephalin clotting time, partial thromboplastin time and one-stage prothrombin time; the small but significant difference in the results of the thrombin clotting time probably reflects the potency of the thrombin used in the test system.

The mean levels in the baby of factor II (54%), factor VII (38%), factor IX (17%) and factor X (39%) were all low and a highly significant difference was present between the value for these clotting factors in the newborn and the mother. In sharp contrast to the low levels of the vitamin K dependent factors in the baby the mean levels of factor V and factor VIII were found to be considerably increased in both the baby and the mother (Figure 24). No significant correlation was present between the levels of factors II, VII, IX and X in the mother and the baby but a highly

significant correlation was found between the maternal and neonatal levels of factor V and factor VIII (Table 43A). In the newborn the platelet count was significantly higher than in the mother but no correlation was present between the neonatal and maternal levels.

The results of the fibrinolytic enzyme system assays are shown in Table 44 and Figure 25. The fibrinogen and plasminogen levels were significantly lower in the baby, the mean values being approximately 50 per cent. of the levels found in the mother. A highly significant difference was found between the euglobulin lysis activity and the urokinase sensitivity test in the newborn and the mother. The euglobulin lysis activity in the baby (Mean 8.9 units) indicated a very high level of fibrinolytic activity in contrast to the diminished fibrinolytic activity present in the mother (Mean 0.39 units).

The urokinase sensitivity test showed a much higher level of urokinase inhibition in the baby (Mean 0.12 units) than was present in the mother (Mean 0.81 units). The level of fibrin/fibrinogen degradation products in the serum was significantly lower in the newborn than in the mother.

DISCUSSION:

Blood coagulation in newborn infants has been the subject of many investigations and low levels of the vitamin K-dependent clotting factors (factors II, VII, IX and X) are well known. Few investigations have been performed on the fibrinolytic activity of the umbilical cord blood and conflicting results have been published. For example, fibrinolytic activity in the cord blood was found to be increased by Cope and Simmons (1958) and Berglund (1958) but not by Phillips and Skrodelis (1958). These discrepancies may be the result of different test methods, the labile nature of plasminogen activator, and the various ways in which the cord blood was obtained. Previous investigations on the haemostatic mechanism in the baby at birth have largely been performed on blood samples taken at varying intervals after the cord was clamped, usually by allowing the blood to drip from the cut end of the cord. Blood obtained in this way is likely to be affected by stasis, trauma to the vessels and contamination by tissue extracts. During the first half-minute after delivery there is a good umbilical blood flow of relatively well-oxygenated venous blood (Dawes, 1968) and sampling of blood

at this time by clean venepuncture is therefore more likely to reflect accurately the coagulation and fibrinolytic status of the newborn baby.

The data presented show striking differences between the coagulation and fibrinolytic systems in the mother and the newborn infant. With the exception of the recalcification time in plastic, the clotting tests in the newborn were significantly longer than the maternal values. Low levels of factor II (prothrombin), VII, IX and X are in keeping with many previous investigations in the newborn (Douglas and Davies, 1955; Fresh et al., 1956; Dyggve , 1958, and others) and these low neonatal levels contrast with the raised levels of factors II, VII and X and the normal level of factor IX in the mother. The lack of correlation and large differences between the maternal and neonatal values confirm a placental barrier to these clotting factors (Nossel et al, 1966; Cade et al, 1969). Contrary to previous reports of normal levels of factors V and VIII in cord blood (Quick et al, 1952; Kasper et al, 1964; Preston, 1964; Cade et al, 1969), this study showed raised levels of factors V and VIII in the newborn and a highly significant correlation

between the maternal and neonatal levels. The high levels of factors V and VIII in the umbilical vein at the time of delivery may perhaps be due to activation of these factors as a result of placental separation, rather than transfer of these factors from the mother. Davie, Hougie and Lundblad (1969) have suggested that the first traces of thrombin that are formed during coagulation have high affinities for factors VIII and V converting them into far more reactive clotting factors. It is likely that the high levels of factors V and VIII found in the cord blood are a transient phenomenon. As was described in Chapter 7 the maternal venous blood draining the placental site showed a sharp, although short-lived rise of these factors immediately after delivery of the baby. Brown and Stalker (1969) have shown in the hamster that thromboplastin material can enter the maternal and foetal circulation during placental separation. The entry of thromboplastin of placental origin into the circulation of the baby and the mother during delivery may explain the raised levels of factors V and VIII and the correlation between the maternal and cord blood levels of these factors. The presence of tissue thromboplastin would also account for the

shortened recalcification time in the cord blood despite the low levels of coagulation factors II, VII, IX and X. Indeed, the very low levels of these factors in the baby at birth may be partly due to their consumption at the time of delivery rather than entirely to hepatic immaturity as generally accepted. The low level of many of the coagulation factors in the newborn indicates the precarious state of the clotting mechanism in the normal neonate and accounts for the increased susceptibility to haemorrhagic complications.

The findings in the components of the fibrinolytic enzyme system show a distinct contrast between the mother and the newborn. The levels of fibrinogen and plasminogen in the baby were approximately half the values found in the maternal blood. Reduced levels of fibrinogen and plasminogen in the newborn have been reported previously (Cope and Mitchell, 1964; Beller et al, 1966; Fisher et al, 1968); the low levels of fibrinogen and plasminogen could be explained by either a reduced production or increased utilization. The euglobulin lysis time indicated a brisk fibrinolytic activity in all the newborn infants, the reverse of the situation of reduced activity in the maternal blood. The cord blood was also much less sensitive

to urokinase-induced lysis than the maternal blood, indicating a very much higher level of urokinase inhibitor in the infant. Kawano and colleagues (1968) showed that the human placenta had a high content of urokinase inhibitor and the placenta may be the source of the increased inhibitory activity in the infant at birth.

The increased fibrinolytic activity in the newborn indicates a high level of plasminogen activator in the baby's circulation immediately after birth and may be the result of stress and transient hypoxia in the infant during vaginal delivery. It is surprising however that the stress and exertion of labour does not influence the reduced fibrinolytic activity in the maternal blood. A high level of plasminogen activator in the baby's circulation may have a protective role for the rapid clearance of intravascular fibrin which could result from the entry of placental thromboplastin into the circulation or from the trauma of child-birth. Increased fibrinolytic activity would however be a potentially hazardous development in the presence of reduced levels of coagulation factors. The high levels of circulating inhibitor which were also demonstrated in the baby would serve to protect the

susceptible plasma proteins and confine the action of plasminogen activator to the digestion of fibrin in accord with Sherry's hypothesis of in vivo fibrinolysis (Sherry et al, 1959). The absence of any increase of the levels of fibrin degradation products in the newborn may be a reflection of the time factor rather than the absence of fibrin deposition; serial measurement of the levels of fibrin degradation products in the infant during the first week of life would be of interest.

In the preceding two chapters evidence was produced that during childbirth an activation of the clotting and fibrinolytic systems takes place in the maternal circulation. This investigation in the newborn suggests that a similar phenomenon takes place in the baby. The most likely explanation appears to be the entry of thromboplastin into the foetal and maternal circulation during delivery. The fibrinolytic enzyme system in the healthy mature newborn however appears to be particularly prepared for the removal of fibrin. Boyd (1966) has shown that widespread intravascular fibrin deposition is frequently found in stillborn infants, particularly following premature separation of the placenta. According to Ambrus (1966) the

level of fibrinolytic activity and circulating plasminogen is considerably reduced in the premature baby. An impaired fibrinolytic response in the premature baby may predispose to the persistence of fibrin which appears to be a predominant feature of hyaline membrane disease.

Studies on the premature infant similar to those reported in this chapter may help to elucidate the problems of haemorrhage and respiratory distress syndrome in the premature baby.

SECTION 3.

THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN COMPLICATIONS OF PREGNANCY

Chapter 9 Fibrin degradation products in complications of pregnancy.

Chapter 10 The coagulation and fibrinolytic mechanisms in abruptio placentae.

Chapter 11 The coagulation and fibrinolytic systems in pre-eclampsia and eclampsia.

Chapter 12. Conclusion and Summary.

CHAPTER 9.

FIBRIN DEGRADATION PRODUCTS IN COMPLICATIONS OF PREGNANCY

The levels of circulating fibrin/fibrinogen degradation products (F. D. P.) may reflect fibrinolysis occurring "in vivo" in response to intravascular fibrin deposition. The part played by fibrinolysis in certain pathological states has been the subject of much dispute. This has been particularly so in complications of pregnancy, where evidence of enhanced fibrinolysis has rarely been demonstrated in the circulating blood by clot lysis or fibrin plate techniques. Chapter 5 described the development of the immunological assay for measuring F. D. P. and the serum levels found in 250 healthy women during uncomplicated pregnancy, labour and the puerperium, and in twelve patients following caesarean section. This chapter presents the results of an investigation of the levels of F. D. P. in the circulation of patients with intrauterine death, abruptio placentae, eclampsia and post-partum haemorrhage.

Patients and Methods :

The obstetric complications investigated consisted of twelve patients with abruptio placentae, four patients with intrauterine death, two patients with eclampsia, and four patients with post-partum haemorrhage. All of the twelve patients with abruptio placentae had a moderate to severe degree of concealed retroplacental haemorrhage as evidenced by the clinical state of the uterus and the presence of retro-placental clot at delivery. In the patients studied after intrauterine death in the third trimester, spontaneous expulsion of the foetus took place within fourteen days of the diagnosis of intrauterine death. The levels of F. D. P. in the two patients with eclampsia were determined following the eclamptic seizures. In the four patients with post-partum haemorrhage, the serious blood loss occurred after placental delivery and was in excess of one litre in each patient.

Blood samples were collected and assayed for serum F. D. P. as described in Chapter 5 (pages 40-42).

RESULTS

The levels of serum F. D. P. found in the obstetric complications studied are shown in Figure 26 and the mean

levels found in the 250 healthy women during normal pregnancy, labour and the puerperium (Chapter 5) are included for comparison. As shown in Figure 26 raised amounts of F.D.P. were found in each complication and substantial levels were detected in the sera of the patients with abruptio placentae. In the two patients with eclampsia in late pregnancy the level of F.D.P. gradually increased over the four days after the eclamptic seizures. In view of the high levels of F.D.P. found in abruptio placentae and eclampsia these conditions were further investigated in detail as described in Chapters 10 and 11. In the first two weeks after intrauterine death the level of F.D.P. was moderately elevated (16 - 48 µg/ml.) and similar levels were found in the four patients examined following post-partum haemorrhage. In both the latter groups no evidence of enhanced fibrinolytic activity was detected in the circulating plasma by the euglobulin clot lysis method.

DISCUSSION

The raised levels of F.D.P. suggest that in these complications of pregnancy both intravascular fibrin formation and fibrin proteolysis are taking place in varying degrees. The

placenta and decidua were observed by Seegers and Schneider (1951) to be particularly rich in clot-promoting substance, and in pregnant women dying soon after abruptio placentae, emboli of fibrin-like material have been found in the pulmonary arterioles and in other organs (Schneider, 1951; Mayer et al, 1954; Johnstone and McCallum, 1956; Beischer, 1961). The activation of the fibrinolytic system is therefore likely to be a direct or indirect protective response to intravascular clotting. High levels of degradation products of fibrin would also be in accord with the concept that lysis of intravascular fibrin is due to local activation of plasminogen within the thrombi (Sherry et al, 1959). The reduced levels of plasminogen activator in pregnancy (Shaper et al, 1965; Nilsson and Kullander, 1967; Bonnar et al, 1969a) may be important in the pathogenesis of such conditions as acute tubular necrosis in association with complications of pregnancy such as abruptio placentae, as low levels of activator could delay the removal of intravascular fibrin in the renal circulation; animal studies have shown that the level of plasminogen activator in the endothelium of renal vessels is decreased during pregnancy (Epstein, 1966).

The substantial levels of F.D.P. are of importance in relation to the defective haemostasis which may present in abruptio

placentae, as the degradation products can interfere with normal fibrin polymerization, the thrombin-fibrinogen reaction, and the functioning of the platelets, with the result that clot formation may be delayed and defective. This aspect is further discussed in Chapter 10 which deals with the behaviour of the coagulation and fibrinolytic systems in abruptio placentae. The increased levels of degradation products in patients following eclamptic seizures also indicated that a more detailed study was required in patients with eclampsia to elucidate the explanation for the abnormal levels of F. D. P. found in the two patients with eclampsia. The investigation of severe pre-eclampsia and eclampsia is described in Chapter 11.

Foetal death in utero is followed by a gradual depletion of clotting factors (Pritchard and Ratnoff, 1955). Intravascular coagulation has been widely incriminated as the usual cause of fibrinogen depletion in patients with a retained foetus (Pritchard, 1959; Hardisty and Ingram, 1965). The report of Lerner and colleagues (1967) that the depleted coagulation factors after intrauterine death were corrected by the administration of heparin alone, without blood or fibrinogen, and the findings in this study of raised serum F. D. P. after intrauterine death are

strong evidence that intravascular coagulation is the mechanism responsible for the depletion of the clotting factors.

The levels of F.D.P. were only slightly elevated in the patients with post-partum haemorrhage in contrast to the patients with abruptio placentae, in whom very high levels were found. Hardaway (1966) has shown experimentally in dogs that haemorrhagic shock is associated with intravascular clotting if hypotension occurs in association with circumstances which favour clotting, such as in pregnancy where high levels of coagulation factors are present. The presence of fibrin thrombi in the pituitary was noted by Sheehan and Murdoch (1938) in their extensive report on necrosis of the anterior pituitary in patients dying after haemorrhagic collapse at delivery. The rapid restoration of the blood volume and correction of hypotension probably limits the progression of intravascular fibrin formation and may explain the rarity of Sheehan's syndrome following post-partum haemorrhage in modern obstetric practice.

In Chapter 5 it was shown that the levels of F.D.P. were increased during normal labour and the puerperium. The demonstration in this investigation of abnormal amounts of F.D.P. suggests that intravascular fibrin deposition and fibrinolysis are

occurring in varying degrees in abruptio placentae, intrauterine death, eclampsia and postpartum haemorrhage. The clinical outcome in these complications of pregnancy probably depends on the balance between clotting and lysis as the condition evolves but both thrombotic vascular occlusion and defective haemostasis are possible sequelae.

CHAPTER 10.

THE BEHAVIOUR OF THE COAGULATION AND FIBRINOLYTIC MECHANISMS IN ABRUPTIO PLACENTAE

Abruptio placentae remains one of the most serious hazards of late pregnancy, and the coagulation defect which may accompany this complication was first described in 1901 by De Lee. Since the report of Dieckmann (1936) the haemostatic defect in abruptio placentae has been ascribed to fibrinogen depletion, but the pathogenesis of the hypofibrinogenaemia has not been adequately elucidated. The main theories advanced in explanation of the fibrinogen depletion and haemorrhagic diathesis associated with abruptio placentae include (1) deposition of fibrin in intrauterine clotting, i.e. loss of fibrinogen into the retroplacental haematoma (Stouffer and Ashworth, 1958; Nilsen, 1963; Willoughby, 1966); (2) liberation into the circulation of tissue thromboplastin which produces intravascular coagulation with consequent fibrin formation (Schneider, 1950, 1951, 1952, 1959; Beischer, 1961); (3) primary activation of the fibrinolytic enzyme system leading to hyperplasminaemia and digestion of circulating fibrinogen

(Moloney et al, 1949; Phillips et al, 1962).

Few documented reports on abruptio placentae are available where specific coagulation studies and assessment of fibrinolytic activity have been performed, largely because of the emergency nature of the situations in which this complication arises and the detailed laboratory procedures involved in many of the assays. This chapter presents the results of a detailed investigation into the coagulation and fibrinolytic mechanisms and platelet function in a group of patients with abruptio placentae.

A complex inter-relationship exists between the two systems of blood coagulation and fibrinolysis, involving a balance between clotting and lysis. Fibrinolytic activity "in vivo" has always been difficult to demonstrate and, as a consequence the part played by fibrinolysis in abruptio placentae has been much disputed. The application of immunological methods to the detection of fibrin/fibrinogen degradation products (F. D. P.) in the circulation has enabled a new approach to be made to the assessment of fibrinolytic activity "in vivo", as these breakdown products provide evidence that proteolytic digestion of fibrin or fibrinogen has probably recently occurred.

Patients and Methods :

Ten patients with abruptio placentae were studied and eight of these had a severe degree of mixed or concealed accidental haemorrhage as evidenced by the clinical condition of the patient, the quantity of retroplacental clot and the delivery of a stillborn infant. The amount of replacement transfusion given to these eight patients varied between 2 and 6 litres of blood. The other two patients had a less serious degree of premature separation of the placenta and the babies were alive at birth.

Blood was collected using plastic syringes, and the plasma samples were obtained by mixing 9 volumes of whole blood and 1 volume of 3.8% sodium citrate and centrifuging at 4°C. Serum samples for F.D.P. assay were obtained by adding the whole blood immediately after venepuncture to tubes containing glass beads and a standard amount of fibrinolytic inhibitor (Aminocaproic acid, 10^{-2} Molar or tranexamic acid 10^{-3} Molar) to arrest fibrinolytic activity in the blood sample subsequent to the collection of the specimen. Examination was also made of blood issuing from the vagina in mixed accidental haemorrhage and of the blood in proximity to the retroplacental clot at delivery of the placenta.

The laboratory methods employed are described in Appendix 3.

Plasma fibrinogen levels were measured as thrombin clottable protein by the method of Ratnoff and Menzie (1965). Plasminogen assays, euglobulin lysis times and thrombin clotting times were carried out as described by McNicol and Douglas (1964). The activity in the euglobulin clot lysis test was expressed in arbitrary units of plasminogen activator, one unit being equivalent to the reciprocal of a lysis time of 300 minutes. Prothrombin was assayed by a one-stage technique using the method of Owren and Aas (1951); factor V by the method of Biggs and Macfarlane (1962); the factors VIII and IX by a one-stage method as described by Breckenridge and Ratnoff (1962). The platelet count was performed according to the method of Dacie (1963); platelet adhesiveness was measured by a modified Hellem's technique (Hirsh et al, 1966) and A. D. P. induced platelet aggregation as described by Born (1962). Fibrin/fibrinogen degradation products were assayed using tanned red cells as described by Merskey et al (1966) with the modifications described previously (Chapter 5; Bonnar et al, 1969c) and the serum was incubated with one-tenth of its volume of thrombin (100 units ml) to remove any residual thrombin-clottable fibrinogen, so that

as far as possible only products immunologically identical to fibrinogen but not clottable by thrombin were measured.

The values for normal pregnancy and labour were determined by an investigation of 20 healthy pregnant women.

RESULTS

In figure 27 the levels of fibrinogen, serum F.D.P., plasminogen and plasminogen activator in the circulating blood in the patients with abruptio placentae are compared with findings in normal labour. The levels recorded are those obtained before any specific therapy had been administered and either before the transfusion of banked blood or shortly after the transfusion had been started. The level of plasma fibrinogen found in normal labour ranged from 230 to 600 mg/100 ml but only one of the patients was within this range and the remainder had levels of between 35 and 200 mg/100 ml. In the eight patients with a severe degree of abruptio placentae the level of serum F.D.P. was substantially above that found in normal labour (2-16μg ml). The plasminogen levels in abruptio placentae were in the range 1.1 to 3.7 casein units ml. Seven of the ten patients had values between 2 and 4 casein units per ml which were much reduced when compared to the level of plasminogen found in normal pregnancy and labour,

although they were within the normal range for non-pregnant subjects (Bonnar et al, 1969a). No significant difference was present between the levels of circulating plasminogen activator, as estimated by the euglobulin clot lysis time, in patients studied during normal labour and in abruptio placentae. As shown in figure 28 considerable overlap was found between the platelet counts of patients with abruptio placentae and those in normal labour.

In one of the ten patients, transient evidence of a systemic proteolytic state was present (figure 29). This patient had a severe mixed accidental haemorrhage, and defective haemostasis was evident from the oozing at the sites of venepuncture. The euglobulin clot lysis time indicated high levels of plasminogen activator in the circulation but two hours later, in this patient, low levels of activator were present. Both the plasminogen and fibrinogen were at very low levels for pregnancy and the serum F. D. P. level was more than 200 times the mean level found in normal labour; the platelet count was also depressed. This patient was given fibrinogen (4 gm over 2 hours) and the level of plasma fibrinogen increased from 80mg to 140mg/100 ml but during the same interval the level of circulating F. D. P. increased

from 600μg to 1200μg/ml serum. Spontaneous delivery occurred five hours after admission and over 500ml of retroplacental clot was present. Post-partum haemostasis was satisfactory although at the time of delivery the platelet count was less than 50,000/ c.mm.

One patient with a severe degree of abruptio placentae and a haemorrhagic state at 34 weeks gestation is of particular interest as she received from her obstetrician no treatment prior to delivery apart from a transfusion of 1 litre of banked blood. In figure 30 the levels of plasma fibrinogen, plasminogen, serum F.D.P. and platelets are shown, and in figure 31 the thrombin clotting time and coagulation factors II, V, VIII and IX. The fibrinogen and plasminogen levels were markedly depressed and the serum F.D.P. level was extremely high at 1280μg/ml. The platelet counts 2 hours and 30 minutes before delivery were 75,000 and 50,000/c.mm. respectively; no aggregation of the platelets could be detected after addition of A.D.P. and platelet adhesiveness to glass was markedly reduced at 20 per cent. The levels of coagulation factors II, V and VIII were found to be abnormally low until after delivery. Following delivery the level of circulating FDP rapidly decreased, and the thrombin

clotting time and the levels of factors II, V and VIII and fibrinogen returned to normal. Reduced platelet adhesiveness and absence of platelet aggregation were found in four patients with abruptio placentae who were investigated prior to the infusion of whole blood.

In figure 32 are shown the spontaneous increase of the plasma fibrinogen and decrease of serum F.D.P. after vaginal delivery in a patient with abruptio placentae. The serial observations performed on this patient indicate that the degradation products were quickly eliminated from the circulation. In the patient shown in figure 32 urine assay for F.D.P. when the serum level was 1280μg/ml showed a level of 40μg/ml. The F.D.P. in the urine may be the result of excretion from the circulating blood or from the lysis of fibrin in the renal vasculature.

In eight patients the blood issuing from the vagina in mixed accidental haemorrhage and the blood surrounding the retroplacental clot after delivery was examined for F.D.P. and plasminogen. The levels of F.D.P. were significantly lower than that found in the circulating blood in the same patients and the plasminogen level was similar to that in the systemic plasma (Table 45).

DISCUSSION

The data presented in this chapter indicate that the depletion of fibrinogen in abruptio placentae is only one part of a complex syndrome involving the coagulation and fibrinolytic mechanisms. The incidence of fibrinogen depletion in abruptio placentae has been reported as varying from 5% (Bourne and Reedel, 1958) to 20% (Feeney, 1955; Hatton, 1961). The present results are in accord with Nilsen (1963) who found that in nearly all cases of abruptio placentae depletion of fibrinogen is present when compared to the levels found in normal labour. In addition to low levels of circulating fibrinogen a decrease in plasminogen and extremely high levels of F. D. P. were found in the presence of diminished systemic fibrinolytic activity. Fibrinolytic activity has in previous studies been assessed solely by clot lysis or fibrin plate techniques and the activity demonstrated in the systemic circulation was interpreted as indicating that fibrinolysis played no part in the syndrome. The substantial levels of F. D. P. in abruptio placentae which have been demonstrated signify that active fibrinolysis is taking place. The lower levels of F. D. P. in the blood issuing from the vagina before delivery and in the blood in proximity to the retroplacental clot after delivery, indicate that local fibrinolytic

activity in the placental bed at the site of the retroplacental clot is not the source of the high levels of the F. D. P. in the circulation.

In the presence of diminished systemic fibrinolytic activity a high level of F. D. P. suggests that these products are most likely the result of lysis of fibrin as opposed to lysis of circulating fibrinogen. Low levels of fibrinogen and plasminogen and large amounts of F. D. P. in the circulation would be consistent with the concept that intravascular coagulation, generalised or localised to specific organs, had been followed by activation of the fibrinolytic mechanism. Removal of fibrin deposits in such circumstances may be mediated through adsorption of circulating plasminogen activator on the fibrin which is then lysed by activation of plasminogen in close association with fibrin. An alternative explanation could be that fibrin is lysed by activator released from underlying endothelium (Todd, 1964; Warren, 1964). The clearance of fibrin from the circulation would seem to be simultaneous with its formation as shown by the sharp fall of fibrin breakdown products after delivery (figure 32); this would explain why evidence of widespread fibrin deposition has rarely been reported in fatal cases. Although such fibrinolytic

activity would appear to be a protective mechanism against vascular occlusion the release of large amounts of degradation products of fibrin into the circulation will have important biological effects.

Circulating F. D. P., particular the early products of digestion of fibrin or fibrinogen, have a complex anticoagulant effect and interfere both with the thrombin-fibrinogen reaction and with the polymerisation of fibrin. "In vitro" F. D. P. also block platelet aggregation and adhesion (Kowalski, 1964; Wilson et al, 1968). As found here in abruptio placentae no A. D. P. induced aggregation of platelets could be demonstrated and platelet adhesiveness was markedly depressed in the presence of high levels of circulating F. D. P. In view of these profound effects of F. D. P. on the coagulation mechanism and platelet function it seems highly probable that breakdown products of fibrin are a major factor in the defective haemostasis of abruptio placentae. These products will also, however, have a beneficial role in inhibiting the process of fibrin deposition in the vascular tree. The friable nature of the clots forming in the presence of breakdown products may be erroneously interpretated as indicating the presence of pathological proteolysis with lysis of fibrinogen in the circulation.

Studies on the amounts of fibrin and haemoglobin in the blood and clots recovered from the uterine cavity of patients with abruptio placentae have shown that intrauterine fibrin deposition is not of major importance in the genesis of the depletion of the circulating fibrinogen (Pritchard and Brekken, 1967). The findings of this study indicate that in abruptio placentae the most likely sequence of events leading to the fibrinogen depletion and defective haemostasis is primary intravascular coagulation most probably as a result of coagulant substances from the placental site entering the circulation as suggested by Schneider (1959). At the sites of fibrin deposition in the vascular compartment, local activation of plasminogen to plasmin would release large amounts of fibrin breakdown products into the circulation. As found in one of the patients (figure 29), the fibrinolytic activity which is usually confined to local action may extend transiently to the general circulation as a systemic proteolytic state (Fletcher et al, 1962).

As the clinical problem in abruptio placentae is short-lived and the great majority of the patients survive, it is very difficult to correlate the specific approach to management in any given patient with the clinical outcome. It is therefore unwise to be dogmatic at the present time about the management of abruptio

placentae. In the context of a generalised haemostatic defect operative interference would seem to be contra-indicated and should be avoided if at all possible. The recent report of Pritchard and Brekken (1967) on 141 cases of abruptio placentae has shown that when vaginal delivery was accomplished without trauma, treatment with fibrinogen was rarely indicated. Administration of fibrinogen is one of the traditional methods of treatment for the defective haemostasis in abruptio placentae. The observation in this study that the F.D.P. level markedly increased after infusion of fibrinogen raises further doubts as to the desirability of this therapy.

Recently Reid and Chan (1968) observed that in five pregnant women bitten by the Malayan pit viper severe hypofibrinogenaemia developed yet no bleeding problems arose. It is now known that the action of this venom is to deplete fibrinogen without affecting other coagulation factors. Depletion of fibrinogen _per se_ is, therefore, likely to be of less importance in the defibrination syndrome than the depletion of the other coagulation factors and the disturbance of the clotting process and platelet function induced by high levels of fibrin breakdown products.

The rapid decrease of the levels of fibrin breakdown products after delivery emphasises that prompt vaginal delivery without

trauma is likely to be the most effective way of restoring the haemostatic mechanism to normal. The decreased levels of factors II, V and VIII, and the disturbance of platelet function indicates that the most rational approach to treatment is the transfusion of fresh whole blood to provide a full complement of coagulation factors and viable platelets, rather than a large quantity of routine bank blood which is deficient in viable platelets and, in some measure, in factors V and VIII. Hypovolaemia and shock have also been shown to predispose to intravascular clotting (Hardaway, 1966) and central venous pressure studies in patients with abruptio placentae have demonstrated that severe hypovolaemia often exists which is not reflected in the arterial blood pressure (O'Driscoll and McCarthy, 1966; De Valera, 1968; Muldoon, 1969). The rapid and adequate correction of the depleted blood volume by monitoring of the central venous pressure may also, therefore, be a factor in limiting the process of intravascular fibrin formation.

The balance of evidence now available which casts more light on the nature of the upset of haemostasis in abruptio placentae suggests that therapy with fibrinolytic inhibitors such

as amino-caproic acid or Trasylol may be potentially hazardous in that such agents will inhibit plasminogen activation in intravascular fibrin deposits and, therefore, they may carry a risk of promoting vascular occlusion and such further complications as renal damage. The appreciation that systemic fibrinolytic activity in pregnancy is already markedly reduced has emphasised the potential hazard of fibrinolytic ihhibitors in this context.

CHAPTER 11

THE COAGULATION AND FIBRINOLYTIC SYSTEMS IN PRE-ECLAMPSIA AND ECLAMPSIA

The aetiology of pre-eclampsia remains obscure. Jeffcoate (1966) summarised many of the pathological factors known to be associated with pre-eclampsia and affirmed that it was a disease of theories. The possible role of the coagulation and fibrinolytic systems was not included and this aspect has received little attention. In some fatal cases of eclampsia, however, a prominent finding has been widespread fibrin deposition. McKay and colleagues (1953) found evidence of widespread thrombus formation at autopsy in 9 out of 10 eclamptic patients, while in patients who survived eclampsia, liver biopsies showed fibrin thrombi. Earlier, Page (1948) and Schneider (1951) suggested that the thrombi found at autopsy in cases of eclampsia resulted from the release of thromboplastic substances from the placental bed.

Recently electron microscopic study of tissue obtained by renal biopsy from patients with pre-eclampsia has revealed glomerular changes which appear to be specific for this disease.

The lesion consists of swelling of the glomerular capillary endothelium, increase of intercapillary cells and deposition of an amorphous fibrinoid material within the cells and beneath the basement membrane. (Pollak and Nettles, 1960; Altchek, 1961). Morris and colleagues (1964) by immunofluorescent techniques showed that the material in the glomeruli was identical to fibrin. Extensive fibrin deposition has also been shown in the spiral arterioles of the placental bed of patients with pre-eclampsia (Dixon and Robertson, 1958) and this was interpreted as being the effect of hypertension.

Lately evidence has been accumulating that the fibrinolytic system may be implicated in the mechanisms which influence blood pressure and blood flow (Niewiarowski, 1968). The physiological role of plasminogen activator is most likely to keep the blood vessels free of fibrin deposits which might interfere with blood flow. In situations where the secretion or action of plasminogen activator is impaired fibrin deposition would be more likely to occur and hypertension may be a compensatory mechanism to overcome increased peripheral

resistance caused by intravascular fibrin.

To the author's knowledge there have been no detailed and serial studies of components of the fibrinolytic enzyme system in a well-defined group of patients with severe pre-eclampsia and eclampsia. The following investigation was undertaken to throw some light on the role of the coagulation and fibrinolytic mechanisms in this complex disease process.

PATIENTS AND METHODS

Ten patients with severe pre-eclampsia between 33 and 37 weeks gestation were studied and two of these patients developed eclampsia. The diagnosis of pre-eclampsia was based upon the following criteria:

(1) a blood pressure of 150/100 mm. Hg. or over, proteinuria of 1 g or over per litre, and clinical oedema.

(2) An uncomplicated pregnancy and normal blood pressure upto the 28th week of pregnancy.

(3) At the postnatal examination six weeks after delivery the patient had a normal blood pressure and no proteinuria.

The control series comprised 10 healthy women with pregnancies uncomplicated by any signs of pre-eclampsia. These patients were matched for age and gestation with the group with pre-eclampsia.

One patient with epilepsy was studied before and after epileptic convulsions to compare the findings with those in the two patients who developedeclamptic fits.

Blood was collected using plastic syringes and plasma samples were obtained by mixing 9 volumes of whole blood to 1 volume of 3.8% sodium citrate and centrifuging at 4°C. Serum samples for assay of fibrin/fibrinogen degradation products (F.D.P.) were obtained by adding the whole blood immediately after venepuncture to tubes containing glass beads and a standard amount of tranexamic acid as described in Appendix 3.

Plasma fibrinogen levels were measured by the method of Ratnoff and Menzie (1965). Plasminogen assays and euglobulin lysis activity were carried out as described by McNicol and Douglas (1964). The urokinase sensitivity test was used as a measure of fibrinolytic inhibitor in the plasma (McNicol et al, 1965). Serum F.D.P. were assayed as

described in Chapter 5 (Bonnar et al, 1969C). Factor VIII was assayed by the one stage method of Breckenridge and Ratnoff (1962). The platelet count was performed according to the method of Dacie (1963). These laboratory methods are described in Appendix 3.

Histological studies were performed on the kidneys of a 24 year old patient with pre-eclampsia who died suddenly 24 hours after eclamptic seizures which developed immediately after delivery. This patient had virtually no antenatal care and was admitted following delivery at home.

Results:

In Figure 33 and Table 46 the levels of fibrinogen, plasminogen and serum F.D.P. in the patients with severe pre-eclampsia are compared with the control patients. No significant difference was found in the levels of fibrinogen or plasminogen but the levels of serum F.D.P. in the patients with pre-eclampsia were significantly higher than in the control patients.

Figure 34 and Table 47 show the findings for the euglobulin lysis activity and the urokinase sensitivity test. Euglobulin lysis activity and urokinase sensitivity were significantly less

in the patients with severe pre-eclampsia, indicating that in these patients lower levels of plasminogen activator and higher levels of inhibitor to urokinase-induced lysis were present in the circulating plasma than in the healthy pregnant women matched for age and gestation.

In Figure 35 and Table 48 the levels of Factor VIII and the platelet count are compared. No significant difference was found in the levels of factor VIII but the platelet counts were significantly lower in the patients with pre-eclampsia than in the control group.

Serial findings in the two patients who developed eclampsia are shown in figures 36 and 37.

The patient in figure 36 was admitted at 34 weeks gestation with severe pre-eclampsia and eclamptic seizures developed the day after admission. Surgical induction was not performed as the cervix was assessed as unfavourable and the obstetrician in charge decided on conservative management; five days after the eclamptic seizures foetal death occurred and the patient delivered spontaneously six days later. Figure 36 shows the euglobulin lysis activity at the lower limit of the normal range until after foetal death;

raised levels of fibrinolytic inhibitor are indicated by the reduced sensitivity to urokinase-induced lysis; the level of serum F.D.P. rose from 2.5 to 60 μg/ml over the three days after the eclamptic seizures and during this period the platelet count was depressed. Only minor changes were found in the levels of fibrinogen, plasminogen and factor VIII.

Figure 37 shows serial results in a patient who developed eclampsia immediately following delivery. Labour was induced at 37 weeks on account of severe pre-eclampsia. The day prior to induction of labour the euglobulin lysis activity was markedly reduced and the change to normal which normally takes place within 1 hour of delivery did not occur until the third day. This delayed return to normal fibrinolytic activity was also found in another two of the patients with pre-eclampsia. As shown in figure 37 the serum F.D.P. level was elevated prior to the onset of labour and in the three days after the eclamptic seizures the F.D.P. markedly increased and far exceeded the levels found in the first week of the normal puerperium; the platelet count was depressed until after delivery.

Figure 38 shows the serial findings in a patient with epilepsy who was admitted at 38 weeks gestation. This patient had no signs of pre-eclampsia and she had two grand mal seizures while in hospital. As shown in figure 38 the level of F.D.P. did not increase following the convulsions, the euglobulin lysis activity remained depressed and reverted to normal levels following delivery, and the urokinase sensitivity test and platelet count were in the range of the control patients.

In figures 39 and 40 are shown the histological sections of the kidney of the patient who died 24 hours after eclamptic seizures. The intralobular arteries and afferent arterioles were occluded by fibrin which was also present in the glomerular tufts.

DISCUSSION

The findings in this study indicate a difference in certain factors concerned with coagulation and fibrinolysis between patients with severe pre-eclampsia and healthy women in late pregnancy. Recent studies which have been reported on fibrinolysis in pre-eclampsia are difficult to compare with the present series because of different assay

methods and the types of patient studied. Nielsen (1969) found no difference in fibrinolytic activity but the fibrin plate technique which he employed does not permit accurate quantitative assessment of fibrinolytic inhibition and the method may be influenced by differing diffusion rates of activators, enzymes and inhibitors. Wardle and Menon (1969) investigated a group of women with hypertension near term or mild pre-eclampsia and found no obvious difference in fibrinolytic activity from patients with normal pregnancies; however, they found higher levels of cryofibrinogen in the hypertensive patients which were interpreted as indicating a low grade intravascular coagulation. The patients in the present study had severe pre-eclampsia and would not be readily comparable to those studied by Wardle and Menon.

The significant differences found in this investigation of pre-eclampsia were :

(1) greater depression of euglobulin lysis activity than present in normal pregnancy;

(2) a higher level of inhibitor to urokinase-induced lysis;

(3) raised levels of serum F.D.P.

(4) reduced platelet counts.

The lowered euglobulin lysis activity reflects reduced levels of circulating plasminogen activator which could result from a reduced production of activator in pre-eclampsia or from the absorption of circulating activator into intravascular fibrin. The increased resistance to urokinase may be a factor in the reduced fibrinolytic activity. In patients with renal disease, McNicol, Barakat and Douglas (1965) found reduced fibrinolytic activity and higher levels of inhibitor to urokinase. The similar changes in severe pre-eclampsia may therefore be related to the renal changes which occur in this condition. The raised levels of circulating F. D. P. most probably reflect local fibrinolysis in areas of intravascular fibrin. A relatively low platelet count in severe pre-eclampsia has been previously reported (Ward and MacArthur 1948; Brain et al 1967) and this could also be the result of increased consumption due to intra-vascular coagulation.

In the two patients with eclampsia the level of serum F. D. P. rose steeply over the three days after the eclamptic seizures but no increase was found in the patient who had epileptic fits. Increased plasma fibrinolytic activity has been found in animals

after electrically induced convulsions (Fantl and Simon, 1948) and in patients following electroshock therapy (Fletcher et al, 1963). Following the convulsions of eclampsia and epilepsy in late pregnancy plasma fibrinolytic activity remained depressed but high levels of F. D. P. appeared in the patients with eclampsia. A possible explanation could be that in the patients with pre-eclampsia intravascular fibrin was present and the convulsions of eclampsia provoked an increase of local fibrinolysis. Schneider (1947) and Page and colleagues (1951) suggested that eclampsia may be a result of sudden intravascular clotting; such an occurrence would produce a rise of F. D. P. in the circulation. Raised levels of F. D. P. may explain why some patients with eclampsia develop bleeding from the gums, skin petechiae and excessive bleeding from surgical incisions.

McKay (1965) has proposed that in pre-eclampsia a low grade process of intravascular coagulation is present and that the convulsions of eclampsia are the result of a sudden agglutination of platelets and the formation of platelet and fibrin thrombi which obstruct the cerebral microcirculation. A small number of publications have supported the assumption of

intravascular coagulation in pre-eclampsia. These include Beller (1964), Vassali and McCluskey (1965) and Hjort and Rapaport (1965). The cause of intravascular coagulation is uncertain. The release of thromboplastin from the placenta, particularly from infarcts and ischaemic areas, has been postulated. McKay and colleagues (1964) suggested that the cause may be coagulation-activating components released from platelets which have been destroyed by adhering to the syncytial trophoblast.

Placental infarcts and intervillous thrombi are a well-known feature of pre-eclampsia and these changes are probably an important factor in the placental insufficiency which occurs in this syndrome. A disturbance of the balance between coagulation and fibrinolysis in localised areas of the vascular compartment - particularly the placental and renal circulation - could explain many of the features of pre-eclampsia. Fibrin deposition in the maternal vessels supplying the placenta particularly the spiral arterioles and consequent impairment of placental blood flow may explain the placental insufficiency which occurs in pre-eclampsia. Similarly fibrin deposition in the renal vasculature could result in glomerular damage and proteinuria. Hypertension may be the result of renal ischaemic

changes or a compensatory response, which is mediated in some unknown way, to the presence of fibrin deposition in the vascular compartment. The extensive fibrin deposition shown in the vessels of the fatal case of eclampsia would account for the development of acute renal failure which may sometimes follow eclampsia.

An important feature of the fibrinolytic inhibition in pregnancy is the abrupt return to normal activity following delivery or intrauterine death. The resolution of the clinical signs of pre-eclampsia following delivery or intra-uterine death is well known. The observation that fibrinolytic activity in some of the patients with pre-eclampsia did not return to normal for 24-48 hours after delivery also indicates a departure from the pattern of fibrinolytic activity found in normal pregnancy.

The demonstration of fibrin by immunofluorescent studies in the renal glomeruli of patients with pre-eclampsia (Morris et al 1964) and the evidence of intravascular fibrin deposition found in this study, raise the question as to the possible therapeutic value of antithrombotic agents such as heparin and dipyrimadole to counteract intravascular coagulation. Such

treatment could reduce thrombotic occlusion in the maternal vasculature supplying the placenta and thereby improve placental perfusion and foetal growth. The value of such therapy would best be evaluated through carefully controlled clinical trials which included a study of placental function and changes in the coagulation and fibrinolytic factors. Fibrinolytic therapy is another possibility but the fibrinolytic agents at present available have considerable drawbacks and investigation in pregnant animals would be required to assess their effect on the foetus and placenta before they could be used in this context.

Whether the changes found in this investigation are related to the pathogenesis of pre-eclampsia or are the effect of the disease process cannot at present be answered. Nevertheless these findings indicate that intravascular fibrin deposition and disordered fibrinolytic activity are a feature of severe pre-eclampsia. Further study of the coagulation and fibrinolytic systems in pre-eclampsia and of the maternal vasculature supplying the placenta may help to elucidate the pathogenesis of this syndrome and help to provide a rational basis for effective treatment.

CHAPTER 12.

CONCLUSIONS AND SUMMARY

The blood coagulation and fibrinolytic systems have a key role in the body's defence mechanism and their principal physiological function appears to be the maintenance of the integrity of the vascular compartment. According to current concepts the two systems may be in a state of dynamic equilibrium, the coagulation system laying down a haemostatic fibrin plug to seal any gaps which may occur in the vascular endothelium, and the fibrinolytic system removing the intra-vascular fibrin when it is no longer required. These physiological systems are thus of major importance in the control of both haemorrhage and thrombosis.

Pregnancy induces extensive changes in the haemostatic mechanism: the gradual increase in the concentration of plasma fibrinogen and factors VII, VIII and X is well-documented and a decrease of fibrinolytic activity is known to develop in late pregnancy. The work described in this thesis extends the knowledge of the changes and functioning of the coagulation and

fibrinolytic systems in both normal and abnormal pregnancy and parturition.

The effect of normal pregnancy on the components of the fibrinolytic enzyme system was investigated and a substantial increase in the plasminogen level was found in the third trimester, the increase occurring pari-passu with a pronounced increase in fibrinogen concentration. The rise in the level of these two components which influence the haemostatic balance in opposite directions would seem to be consistent with the concept of a dynamic equilibrium between clotting and lysis. In late pregnancy and during labour the level of plasminogen activator in the plasma was greatly decreased, whereas a normal level was present in the first week of the puerperium. The mechanisms whereby the alterations in coagulation and fibrinolysis are brought about in pregnancy are unknown. The changes evident in the circulating plasma imply that the haemostatic system is deviated towards an enhanced capacity to form fibrin and a diminished ability to lyse fibrin. During normal labour, however, the level of fibrin/fibrinogen degradation products (F. D. P.) sharply increased indicating that active fibrinolysis was taking place.

As systemic fibrinolytic activity is consistently diminished during labour, the increased levels of F.D.P. may be explained by the lysis of fibrin in localised areas of the circulation, such as in the maternal vasculature supplying the placenta. In restricted areas of the circulation the fibrinolytic process may operate by a local release of plasminogen activator which to a great extent may be independent of the level of systemic fibrinolytic activity.

During normal childbirth distinct changes in keeping with activation of the clotting mechanism take place during and immediately after placental separation. In the sequential study of normal delivery, shortening of the clotting test results, a sharp increase of factors VIII and V, and a decrease of plasma fibrinogen were found as the placenta separated. Within one hour of normal delivery the levels of serum F.D.P. increased and plasma fibrinolytic activity returned to normal non-pregnant levels. The simultaneous study of the changes in the uterine circulation and peripheral blood at caesarean section confirmed a striking activation of the clotting mechanism during placental separation. The changes in the coagulation tests in the uterine circulation were largely a substantial amplification of those found in the peripheral blood at normal childbirth. The potency

of the mechanisms for keeping the clotting of blood a local phenomenon is shown by the fact that the pronounced changes in the uterine vein were short-lived and had almost disappeared by the time the blood reached the peripheral circulation.

The concurrent findings in the blood clotting and fibrinolytic systems indicate that during parturition the haemostatic mechanism appears to play an essential part in controlling uterine haemorrhage. The increased levels of clotting factors during normal pregnancy probably represent, therefore, a physiological development to provide for the rapid and effective haemostasis which is required during parturition.

In the early puerperium a secondary increase took place in the plasma fibrinogen, factor VIII remained elevated, and the platelet count showed a pronounced rise. These changes may be a response to the utilisation of these factors during placental separation and they are likely to predispose to thrombo-embolic complications, which are much more common in the puerperium than during pregnancy. If, as would seem likely, the stimulus to the puerperal changes, especially of

the platelets, occurs during parturition it is possible that further knowledge may enable measures to be taken at childbirth which prevent the postpartum changes in the haemostatic mechanisms and thereby reduce the hazard of thrombo-embolism.

The findings in the coagulation and fibrinolytic systems in the newborn are in sharp contrast to those in the mother. Low levels of Vitamin K dependent clotting factors are well-known. Raised levels of factors V and VIII were found in the newborn and a highly significant correlation between the maternal and neonatal values, suggesting that during normal delivery an activation of the clotting system takes place in the baby's blood as well as in the maternal blood, due apparently to the entry of thromboplastin into the foetal and maternal circulation during placental separation. Fibrinolytic activity was greatly increased in the newborn contrasting with the diminished activity in the maternal blood; this could be the effect of stress and hypoxia in the infant during labour and delivery and may have a protective role for the rapid clearance of intravascular fibrin. The high levels of circulating inhibitor which were also found in the baby would serve to protect susceptible plasma proteins and confine the results of plasminogen

activation to the digestion of fibrin. Further studies of the functioning of the coagulation and fibrinolytic systems particularly in the premature infant may help to throw new light on such complications as haemorrhagic disease of the newborn and hyaline membrane disease.

The complication of pregnancy most likely to be accompanied by defective haemostasis is abruptio placentae. Detailed studies of patients with abruptio placentae showed that fibrinogen depletion was only one aspect of the disturbance of the haemostatic mechanism. Low fibrinogen levels were accompanied by a decrease of plasminogen and coagulation factors II, V and VIII. Substantial levels of serum F.D.P. were found in the presence of diminished systemic fibrinolytic activity, which suggests that these products are the result of local lysis of intravascular fibrin deposits rather than circulating fibrinogen. Platelet aggregation was markedly reduced in the presence of high levels of circulating F.D.P. In view of the profound effect of F.D.P. on the clotting mechanism and platelet function their presence in high levels is likely to be a major factor in the defective haemostasis of abruptio placentae.

The sequence of events leading to the haemostatic defect in abruptio placentae would appear to be a primary intravascular clotting and a simultaneous activation of the fibrinolytic system to remove the fibrin in the vascular compartment. The activation of the clotting mechanism which is confined to the uterine circulation during normal placental separation presumably extends into the general circulation in patients with premature separation of the placenta. The administration of fibrinogen was found to result in a sharp increase of the level of circulating F. D. P. Correction of the hypovolaemia by fresh whole blood to provide a full complement of haemostatic components including platelets seems to be the most rational approach to therapy, with monitoring of the central venous pressure as a guide to the adequacy of blood volume replacement. On the basis of these findings the use of fibrinolytic inhibitors may be potentially hazardous in promoting vascular occlusion. Vaginal delivery was shown to be followed by rapid fall in the level of F. D. P. and a spontaneous recovery of the depleted clotting factors; early delivery without trauma is therefore the most effective way of restoring the haemostatic process to normal.

Although the maternal mortality from pre-eclampsia has been greatly reduced, this unexplained complication of pregnancy is still associated with a high perinatal loss. Investigation of a group of patients with severe pre-eclampsia revealed evidence of a deviation from the findings in the coagulation and fibrinolytic systems during normal pregnancy. The effective level of plasminogen activator in the plasma was lower than found in normal pregnancy, a higher level of inhibitor to urokinase was present, the level of serum F. D. P. was increased and the platelet count reduced. The changes could be explained by intravascular fibrin deposition as a result of a disturbance of the balance between clotting and fibrinolysis in localised areas of the vascular compartment. Such a derangement, particularly in the maternal vascular supply to the placenta and in the renal circulation, could produce many of the features of pre-eclampsia. Narrowing and occlusion of the spiral arterioles supplying the placenta, by mural thrombi, would explain the impairment of placental blood flow and the development of placental infarction and ischaemia which occur in this syndrome. A similar phenomenon in the renal circulation could account for hypertension and proteinuria.

Serial studies following eclampsia showed a steep rise of the serum F. D. P. but no such increase was found after grand mal seizures in late pregnancy. Eclamptic and epileptic convulsions in pregnancy did not provoke any increase of plasma fibrinolytic activity. Histological examination of the kidneys in a fatal case of eclampsia showed evidence of extensive fibrin deposition in the intra-lobular arteries, afferent arterioles, and the glomerular capillaries. The rise of F. D. P. following eclampsia probably therefore reflects local fibrinolytic activity occurring in response to extensive intravascular fibrin deposition.

The evidence of intravascular coagulation in pre-eclampsia raises the possibility of using antithrombotic agents such as heparin to prevent fibrin deposition in the maternal circulation. A carefully controlled clinical trial is now in progress to determine the value of such therapy in improving placental blood flow, placental function, and foetal growth in pregnancies complicated by pre-eclampsia and foetal growth retardation.

The factors controlling local fibrinolytic activity are obviously of importance for the removal of intravascular fibrin deposits. The placenta is a potent source of both fibrinolytic inhibitor and thromboplastin. The application of the techniques

for measuring tissue fibrinolytic activity to the endothelium in the maternal vasculature supplying the placenta in both normal pregnancy and pregnancy complicated by pre-eclampsia and placental insufficiency would be of interest.

Much of the functioning of the clotting and lytic systems in normal and abnormal pregnancy remains to be elucidated and this area of research presents a new challenge to the obstetrician. The number of therapeutic agents which influence the clotting and fibrinolytic mechanisms is steadily increasing. Further knowledge of the physiology and pathology of these systems in pregnancy may define a use for these agents and open up a new field of rational treatment for several of the hazards inherent in pregnancy.

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M.D. THESIS SUMMARY.

THE BLOOD COAGULATION AND FIBRINOLYTIC SYSTEMS IN NORMAL AND ABNORMAL PREGNANCY.

by

John Bonnar.

The introductorysection of the thesis presents a brief historical review of the development of knowledge of the effect of pregnancy on the haemostatic mechanism and gives an account of current concepts of the components and functions of the fibrinolytic enzyme system and the blood clotting mechanism.

The effect of pregnancy and parturition on the components of the fibrinolytic enzyme system was investigated by serial studies of healthy women during normal pregnancy, labour and the puerperium. A marked deviation from the findings in normal non-pregnant subjects was found to develop during pregnancy. A substantial increase in the plasminogen level was found in the third trimester, the increase occurring pari-passu with a pronounced increase in the plasma fibrinogen concentration. In late pregnancy and during labour the level of plasminogen activator in the plasma was greatly decreased, whereas a normal level was present in the first week of the puerperium.

A haemagglutination inhibition assay using sensitized sheep red cells was used to measure the level of fibrinogen/fibrin degradation products (F.D.P.) during normal pregnancy, labour and the puerperium. A sharp increase in the level of F.D.P. was found to

occur during labour and in the puerperium, indicating that active fibrinolysis was taking place at these times.

The effect of the process of childbirth and placental separation on the haemostatic mechanism was investigated by a detailed serial study of the coagulation and fibrinolytic systems during and after delivery. Shortening of clotting tests - recalcification time in plastic, kaolin-cephalin clotting time and partial thromboplastin time - a sharp increase of factors VIII and V, and a decrease of plasma fibrinogen were found as the placenta separated. Within one hour of normal delivery the levels of serum F.D.P. increased and plasma fibrinolytic activity returned to normal non-pregnant levels. The effect of placental separation was further investigated by simultaneous study of the changes in the uterine circulation and peripheral blood at caesarean section while the placenta was separating. A striking activation of the clotting mechanism was found in the uterine circulation during placental separation. The changes in the coagulation tests in the uterine circulation were largely a substantial amplification of those found in the peripheral blood at normal childbirth. The concurrent findings in the blood clotting and fibrinolytic systems suggest that during parturition the haemostatic mechanism plays an

essential role in controlling uterine haemorrhage. In the early puerperium a secondary increase took place in the level of plasma fibrinogen, factor VIII remained elevated and the platelet count showed a pronounced rise. These changes are probably a response to the utilization of these factors during placental separation and are likely to predispose to thrombo-embolic complications.

The coagulation and fibrinolytic systems in the newborn were studied in cord blood taken immediately the baby was delivered. The findings in the newborn were in sharp contrast to those in the mother. Low levels of Vitamin K dependent clotting factors were confirmed but raised levels of factors V and VIII were found and a highly significant correlation between the maternal and neonatal values. Fibrinolytic activity was greatly increased in the newborn contrasting with the diminished activity in the maternal blood. High levels of circulating inhibitor were also found in the cord blood. The findings in the cord blood suggest that during normal delivery an activation of the clotting mechanism takes place in the baby's blood as well as in the maternal blood, due apparently to the entry of thromboplastin into the foetal and maternal circulation during placental separation.

Abruptio placentae is the complication of pregnancy most likely to be accompanied by defective haemostasis. Detailed studies of patients with abruptio placentae showed that low fibrinogen levels were accompanied by a decrease of plasminogen and depletion of coagulation factors II, V and VIII. Substantial levels of serum F.D.P. were found in the presence of diminished systemic fibrinolytic activity. As F.D.P. have a profound effect on the clotting mechanism and platelet function their presence in high levels is likely to be a major factor in the defective haemostasis of abruptio placentae. The findings suggest that the activation of the clotting mechanism which is confined to the uterine circulation during normal placental separation extends into the general circulation in patients with premature separation of the placenta.

Investigation of a group of patients with severe pre-eclampsia revealed evidence of a deviation from the findings in the coagulation and fibrinolytic systems during normal pregnancy. The effective level of plasminogen activator in the plasma was lower than found in normal pregnancy, a higher level of inhibitor to urokinase was present, the level of serum F.D.P. was increased and the platelet count reduced. The changes found in patients with severe pre-eclampsia are in keeping with intravascular

fibrin deposition. Serial studies following eclampsia showed a steep rise of the serum F.D.P. but no such increase was found after grand mal seizures in late pregnancy. Histological examination of the kidneys from a fatal case of eclampsia showed extensive fibrin deposition in the renal vasculature. A disturbance of the balance between clotting and fibrinolysis in local areas such as the uterine and renal circulations could explain many of the features of the syndrome of pre-eclampsia. The evidence of intravascular coagulation in pre-eclampsia raises the possibility of using anti-thrombotic agents to counteract fibrin deposition in the maternal circulation.

Further knowledge of the physiology and pathology of these coagulation and fibrinolytic systems in pregnancy may define a rational use for agents influencing these mechanisms and open up a new field of treatment for several of the hazards inherent in pregnancy.

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FORM OF APPLICATION FOR DEGREE OF M.D.

TITLE OF THESIS: The Coagulation and Fibrinolytic Systems in Normal and Abnormal Pregnancy.

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DATE OF BIRTH: 12.7.34 **DATE OF GRADUATION AS M.B.,Ch.B. (GLASGOW)** July, 1958.

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M.R.C.O.G.	Royal College of Obstetricians and Gynaecologists	July, 1963

MEDICAL APPOINTMENTS HELD SINCE GRADUATION

Designation of Post	where held	from	to
Senior Registrar (Obstet. & Gynaecology)	Glasgow Teaching Hospitals	1964	1969
First Assistant	Nuffield Dept. of Obstet. & Gynae. University of Oxford	1969	to date

NAME AND ADDRESS OF GENERAL PRACTICE, HOSPITAL, DEPARTMENT, LABORATORY OR OTHER INSTITUTION WHERE WORK FOR THIS THESIS WAS UNDERTAKEN

University Dept. of Medicine, Royal Infirmary, Glasgow.

Nuffield Dept. of Obstetrics and Gynaecology, University of Oxford.

DECLARATION I declare that the work has been done and the thesis composed by myself, and that the books and papers cited were all consulted by me personally, unless it is otherwise stated.

(**NOTE:** where material based on work undertaken in collaboration with others is included in the thesis a further and separate statement must be submitted clearly defining the candidate's individual contribution)

DATE: 15th Sept 70 **SIGNED:** John Bonnar

CERTIFICATION:

I hereby certify that the above named candidate for the degree of M.D. has been engaged since graduation for at least one year either in scientific work bearing directly on his profession or in the practise of Medicine.

PERIOD CERTIFIED: 1964 - 1969 **SIGNED:** A S Douglas.

DATE: 15.9.70. **ADDRESS:** University Dept of medicine Royal Infirmary, Glasgow.

POSITION: Professor of Medicine

VOLUME II

APPENDIX 1 - Figures 1 - 40

APPENDIX 2 - Tables 1 - 48

APPENDIX 3 - Methods

APPENDIX I.

Figures 1 - 40

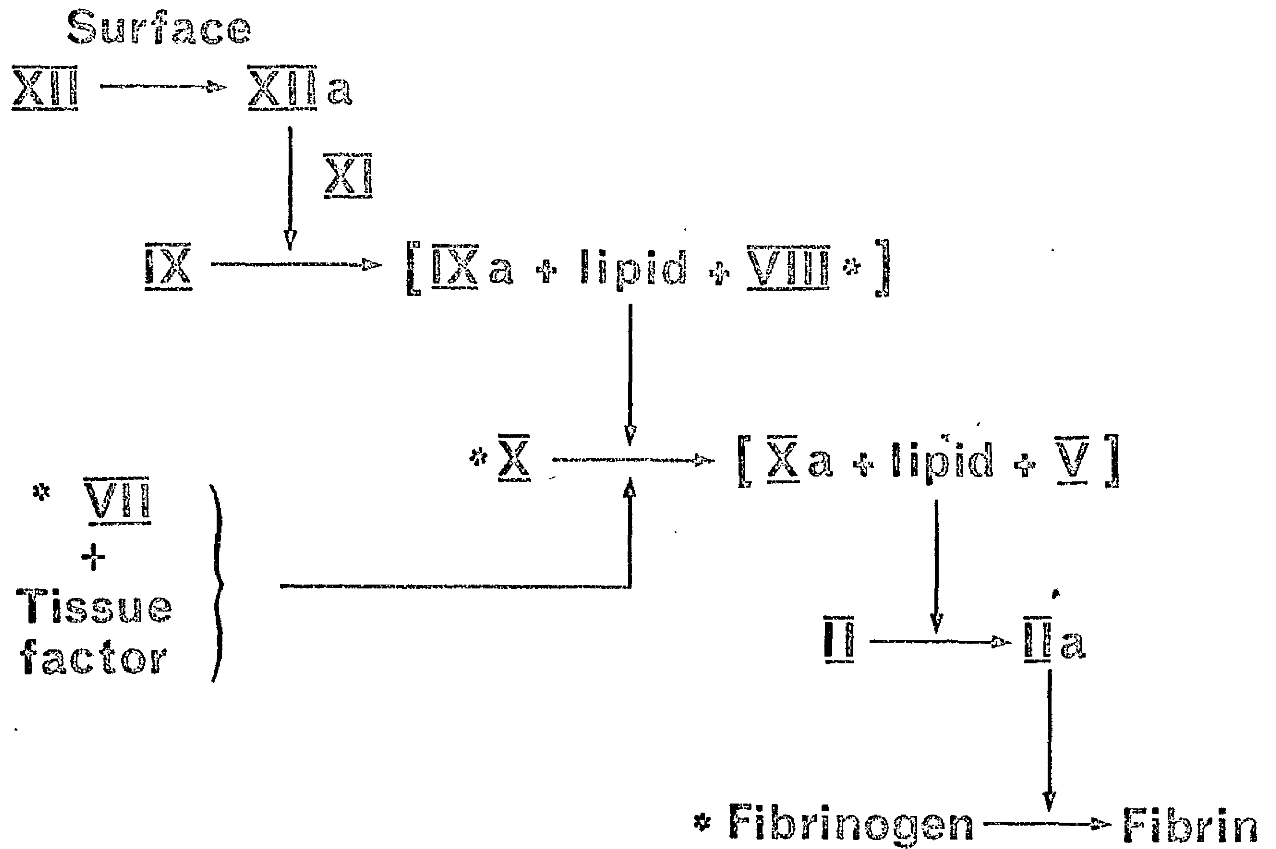

Figure 1

The 'cascade' or 'waterfall' hypothesis of the sequence of reactions in blood coagulation as modified by Esnouf (1968). Each clotting factor or pro-enzyme is represented by a Roman numeral in accordance with international agreement; The letter 'a' added to the numeral represents the 'activated' factor or enzyme. The factors marked with an asterisk are those which have been shown to be increased in pregnancy.

PROTEOLYTIC STEP	FIBRINOGEN ⟶ FIBRIN MONOMER + PEPTIDES
POLYMERISATION STEP	FIBRIN MONOMERS ⟶ FIBRIN POLYMER
GELATION & STABILISATION	FIBRIN POLYMERS ⟶ FIBRIN (Factor XIII ↓; Ca^{++} ↑)

Figure 2

The stages in the conversion of fibrinogen to fibrin by thrombin.

THE FIBRINOLYTIC ENZYME SYSTEM

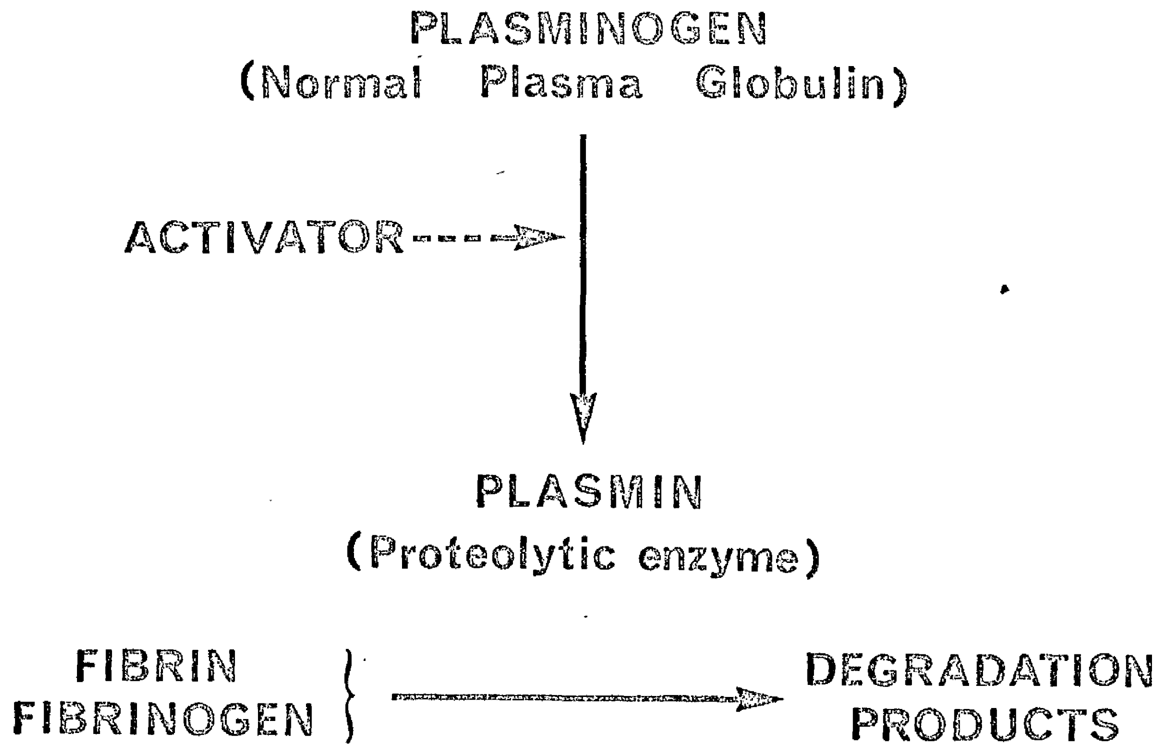

Figure 3

The basic components of the fibrinolytic enzyme system or plasminogen - plasmin system.

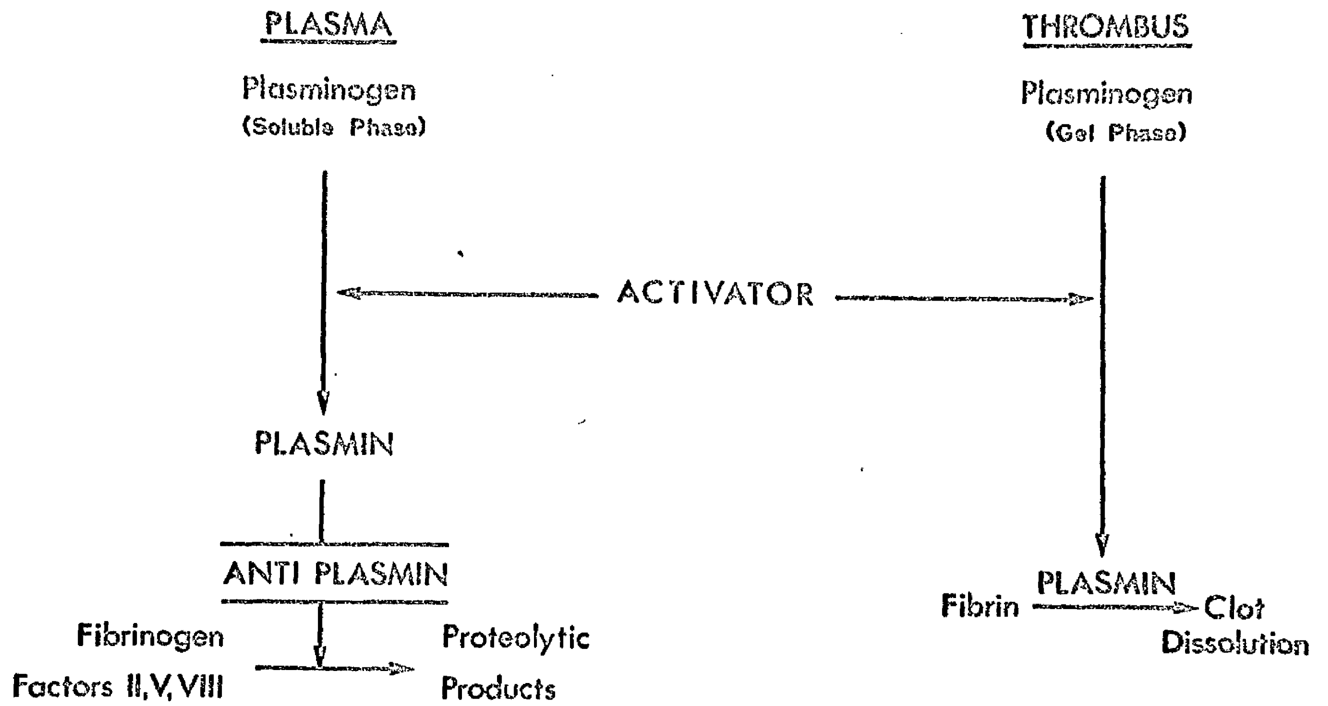

Figure 4

The physiological dual phase mechanism of plasminogen activation in vivo as postulated by Sherry et al (1959).

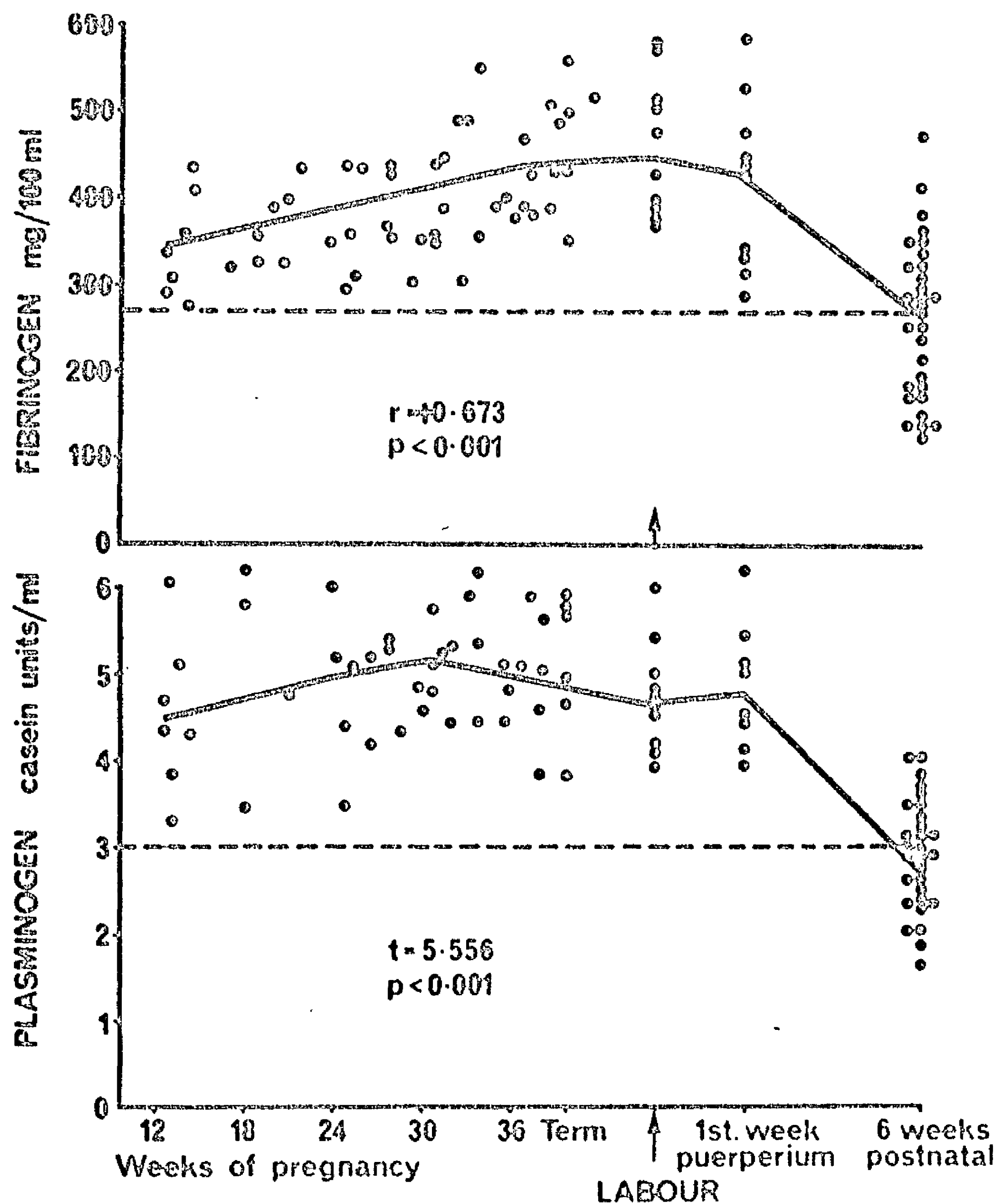

Figure 5

The levels of fibrinogen and plasminogen in pregnancy, labour and the first week of the puerperium compared with the fibrinogen and plasminogen levels in the control group. The figure is prepared from Tables 2, 3 and 8.

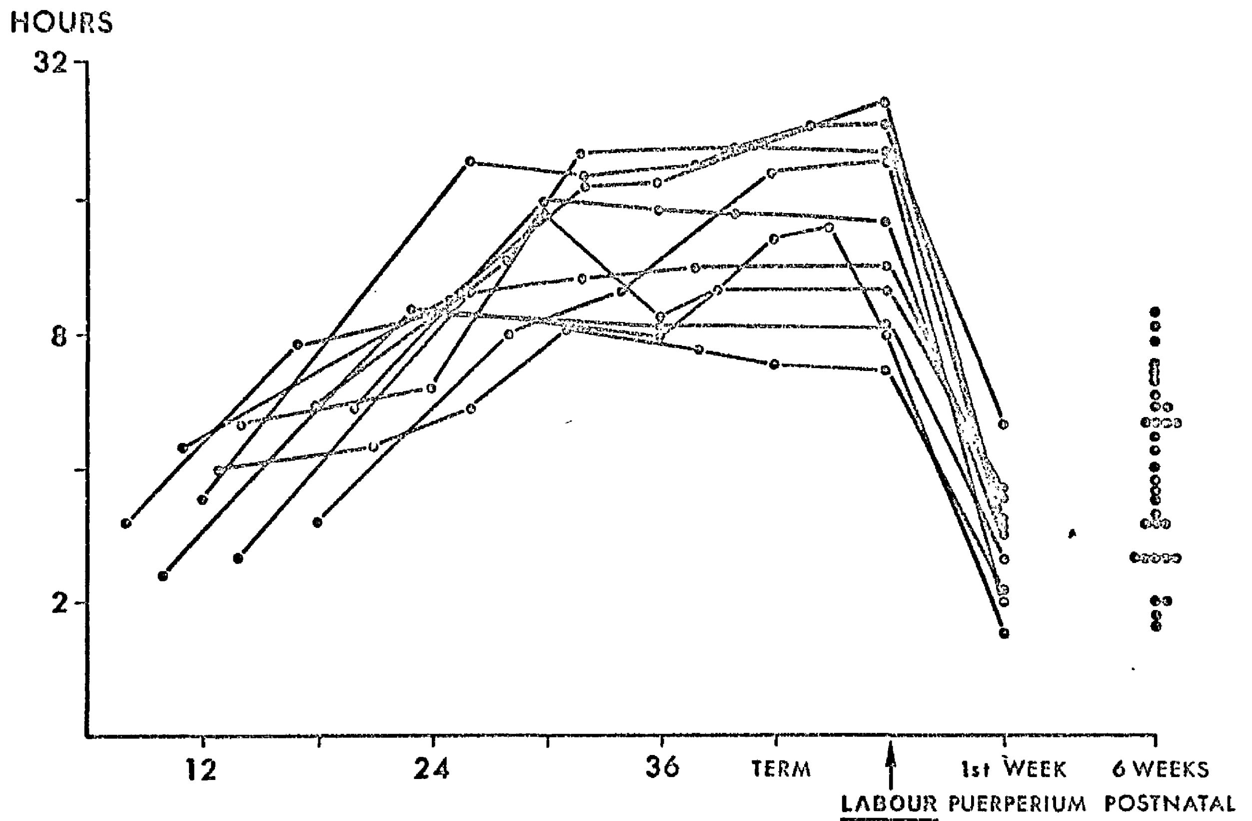

Figure 6

The euglobulin lysis times recorded during pregnancy, labour and the first weeks of the puerperium compared with the findings in the control group (semi-log. scale).

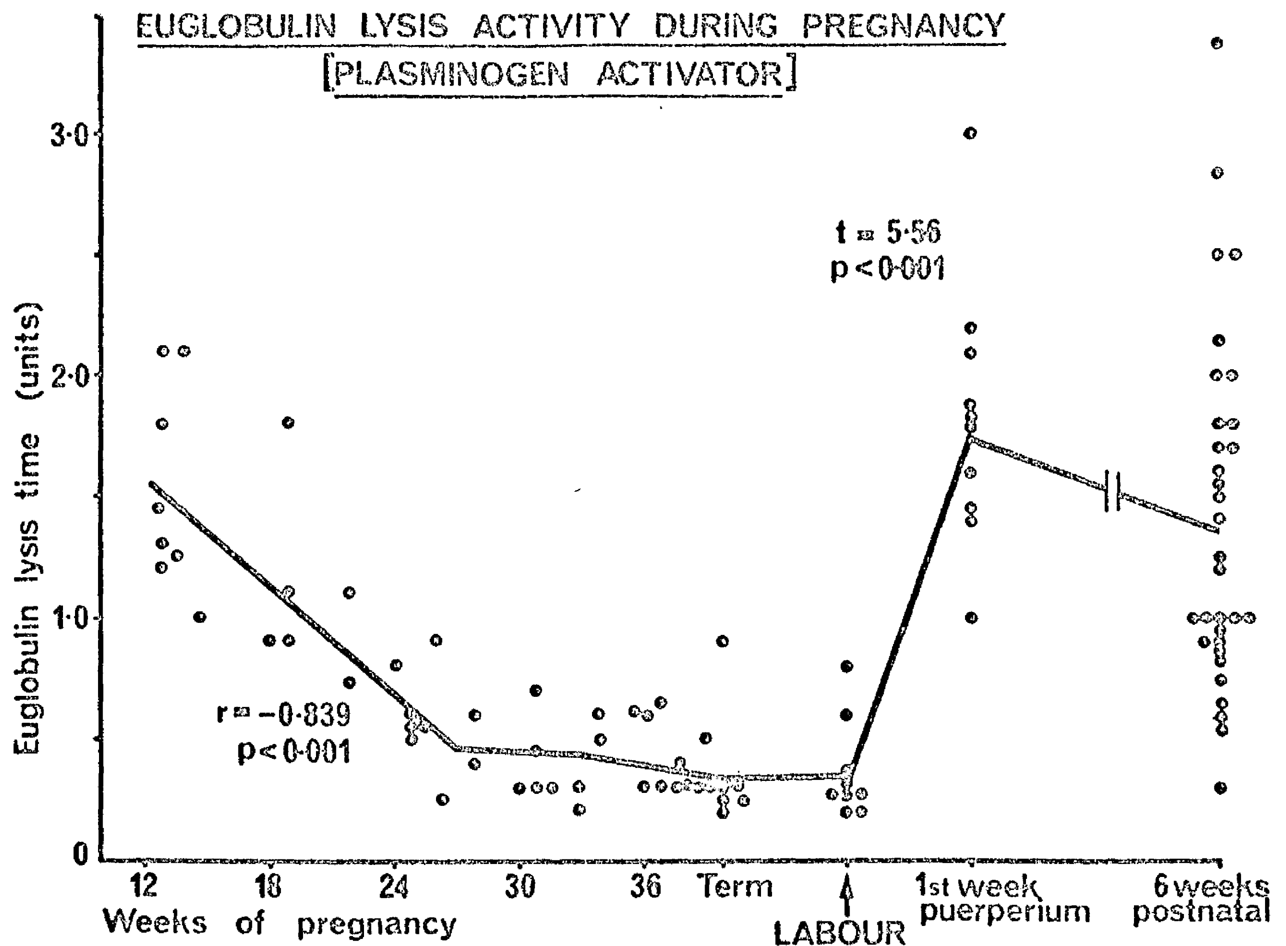

Figure 7

The euglobulin lysis time expressed as units of activity during pregnancy, labour and the first week of the puerperium compared with the findings in the control group. The figure is prepared from Tables 4 and 8.

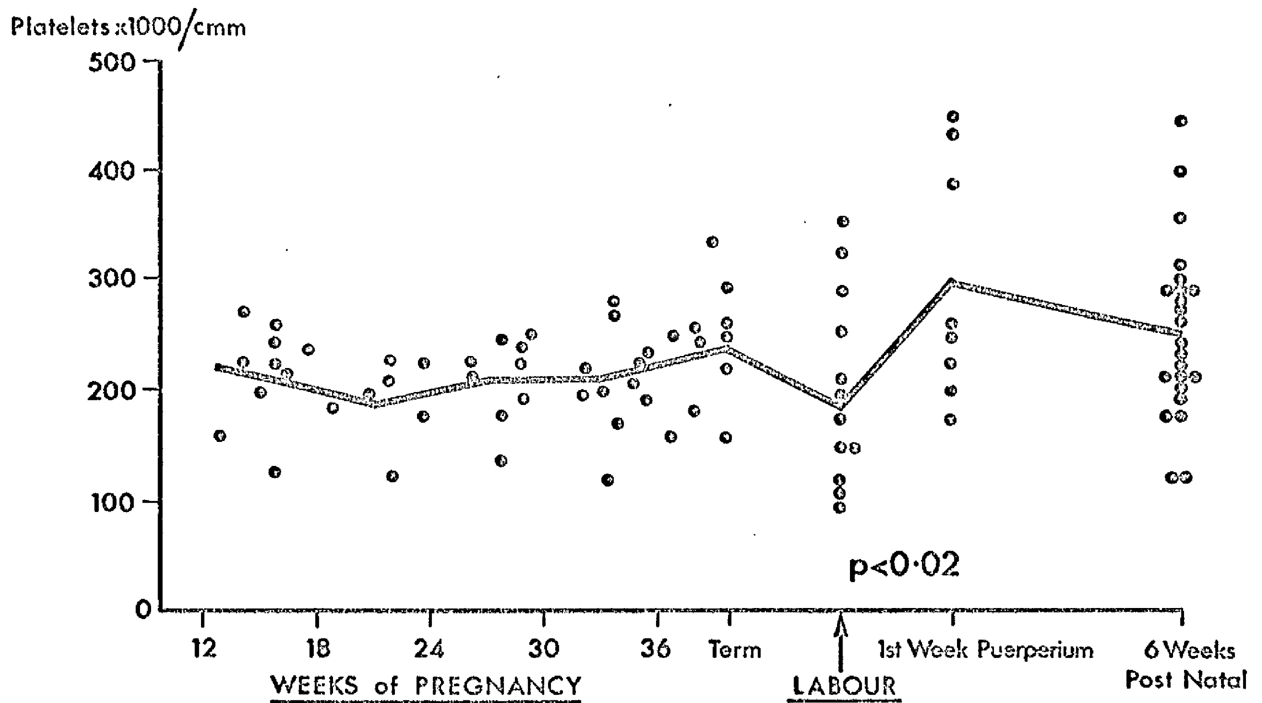

Figure 8

The platelet count during pregnancy, labour and the first week of the puerperium compared with the platelet count in the control group. The figure is prepared from Tables 7 and 8.

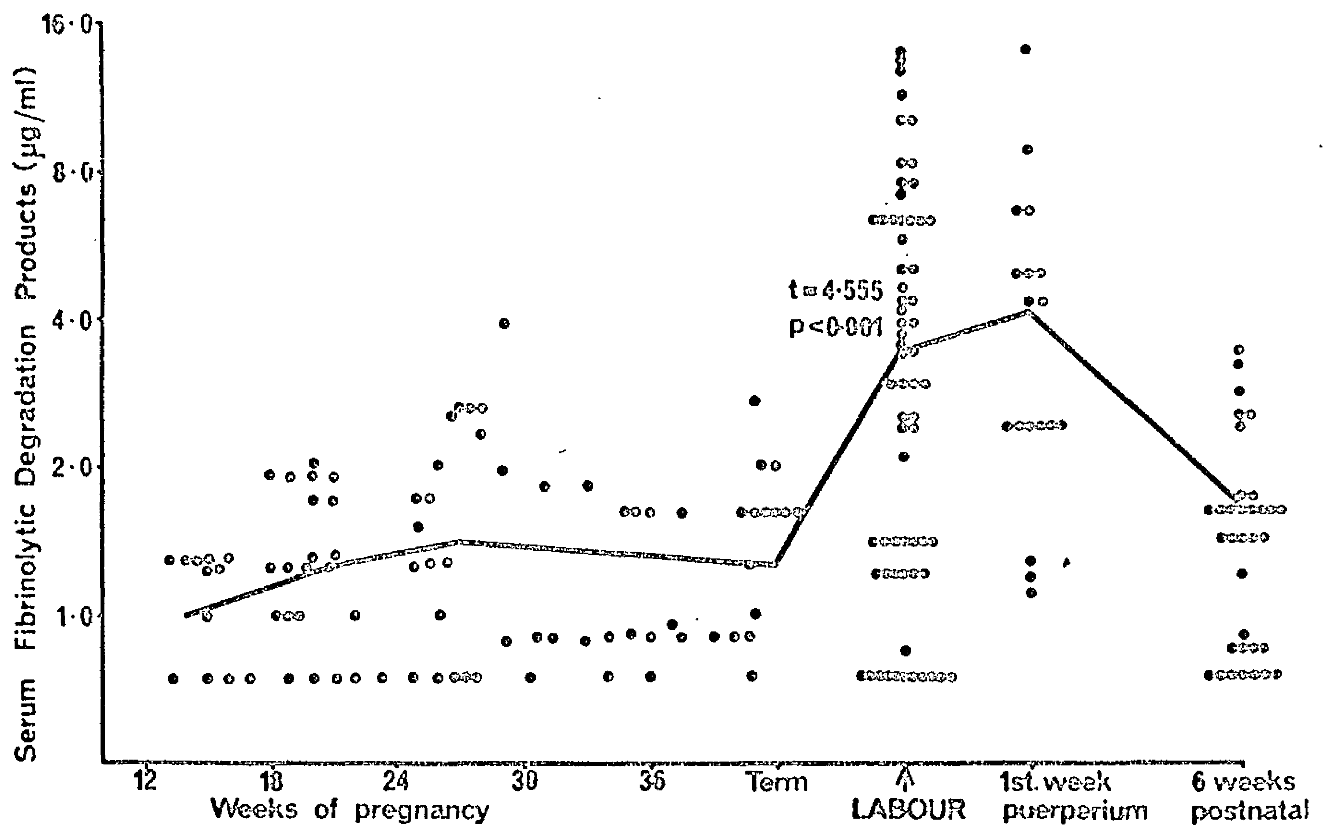

Figure 9

The levels of fibrin / fibrinogen degradation products found in the serum during pregnancy, labour and the puerperium (semi-log. scale). The mean values and standard deviations are shown in Table 9.

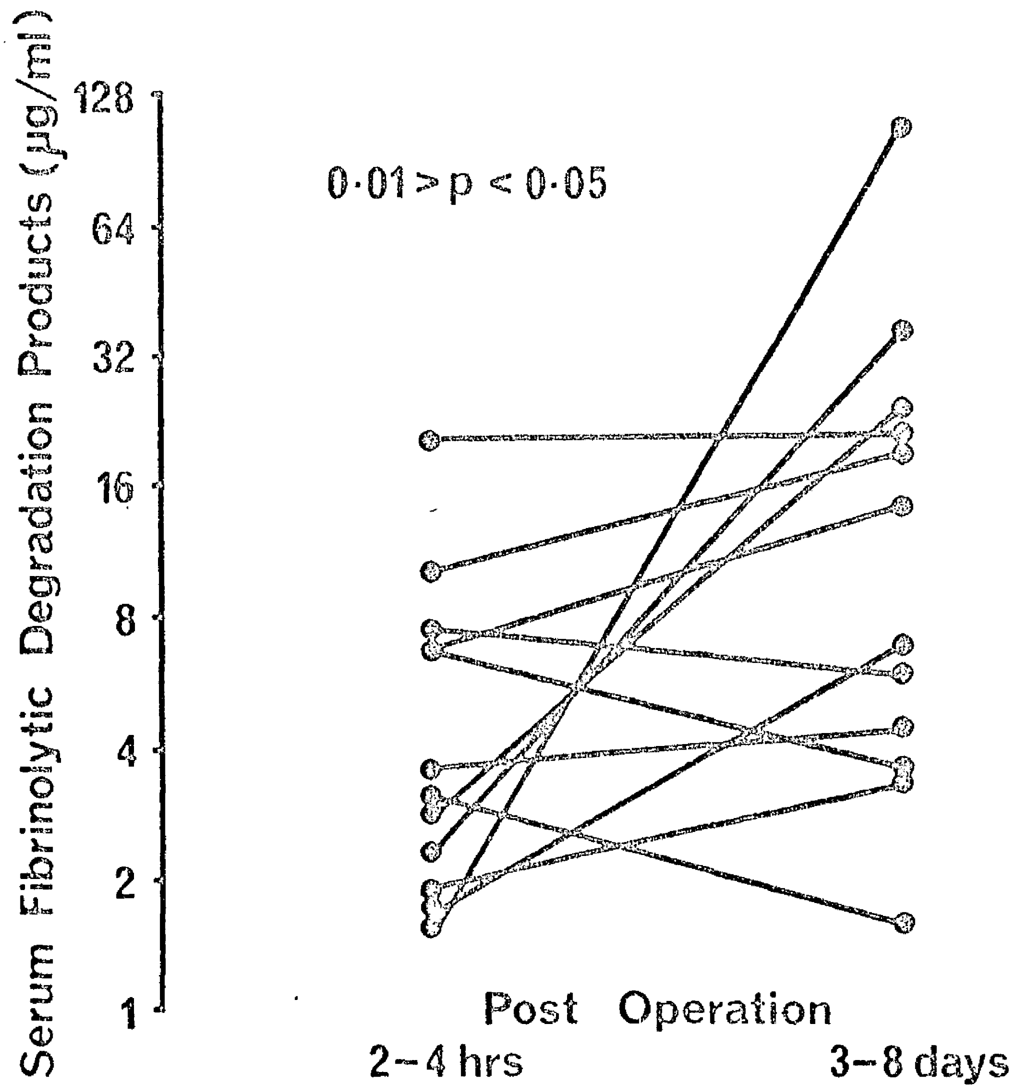

Figure 10

The levels of fibrin / fibrinogen degradation products in the serum of 12 patients tested 2 - 4 hours after operation and again 3 - 8 days after operation. The figure is prepared from Table 10.

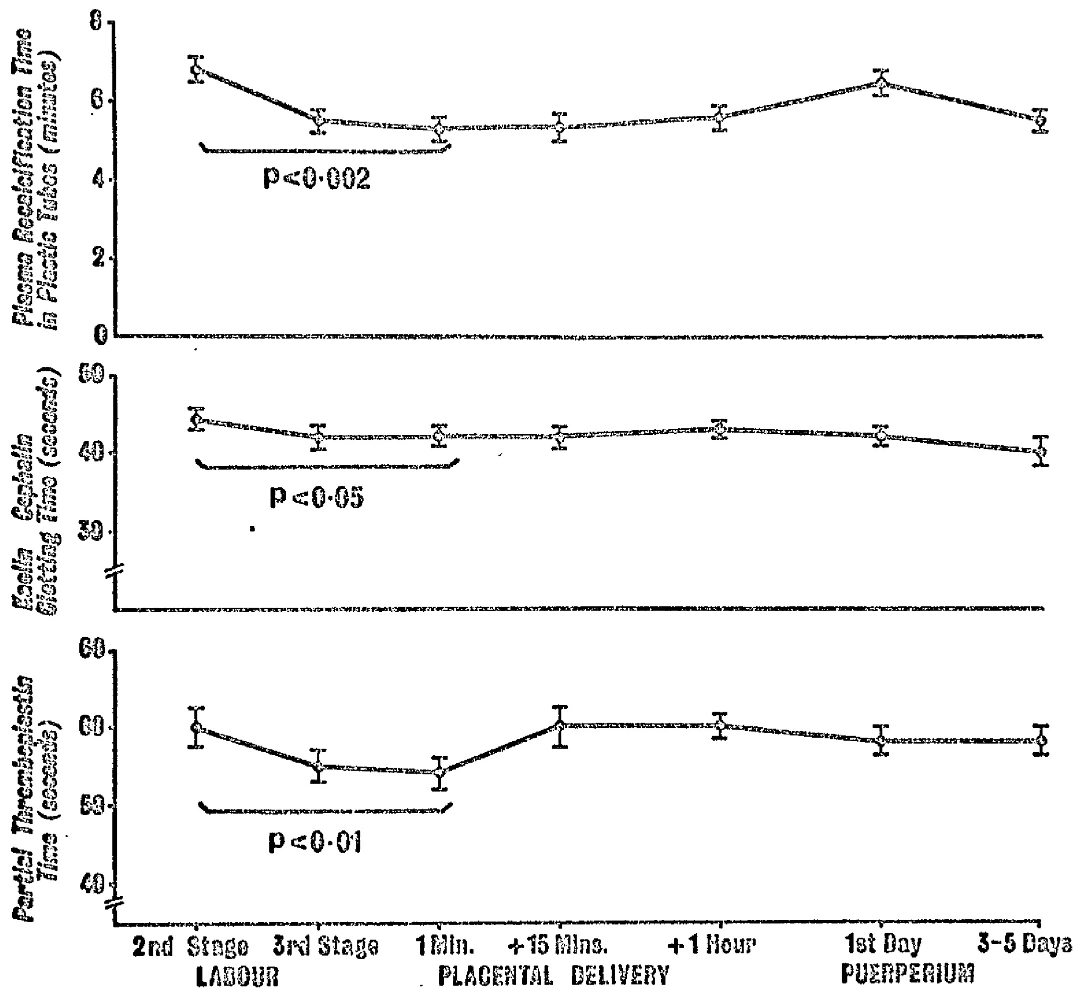

Figure 11

The effect of normal delivery on the plasma recalcification time in plastic tubes, the kaolin - cephalin clotting time and the partial thromboplastin time (mean values and standard error of the mean). The significant shortening of these clotting tests during the third stage and after placental delivery is shown. The figure is prepared from Tables 11, 12 and 13.

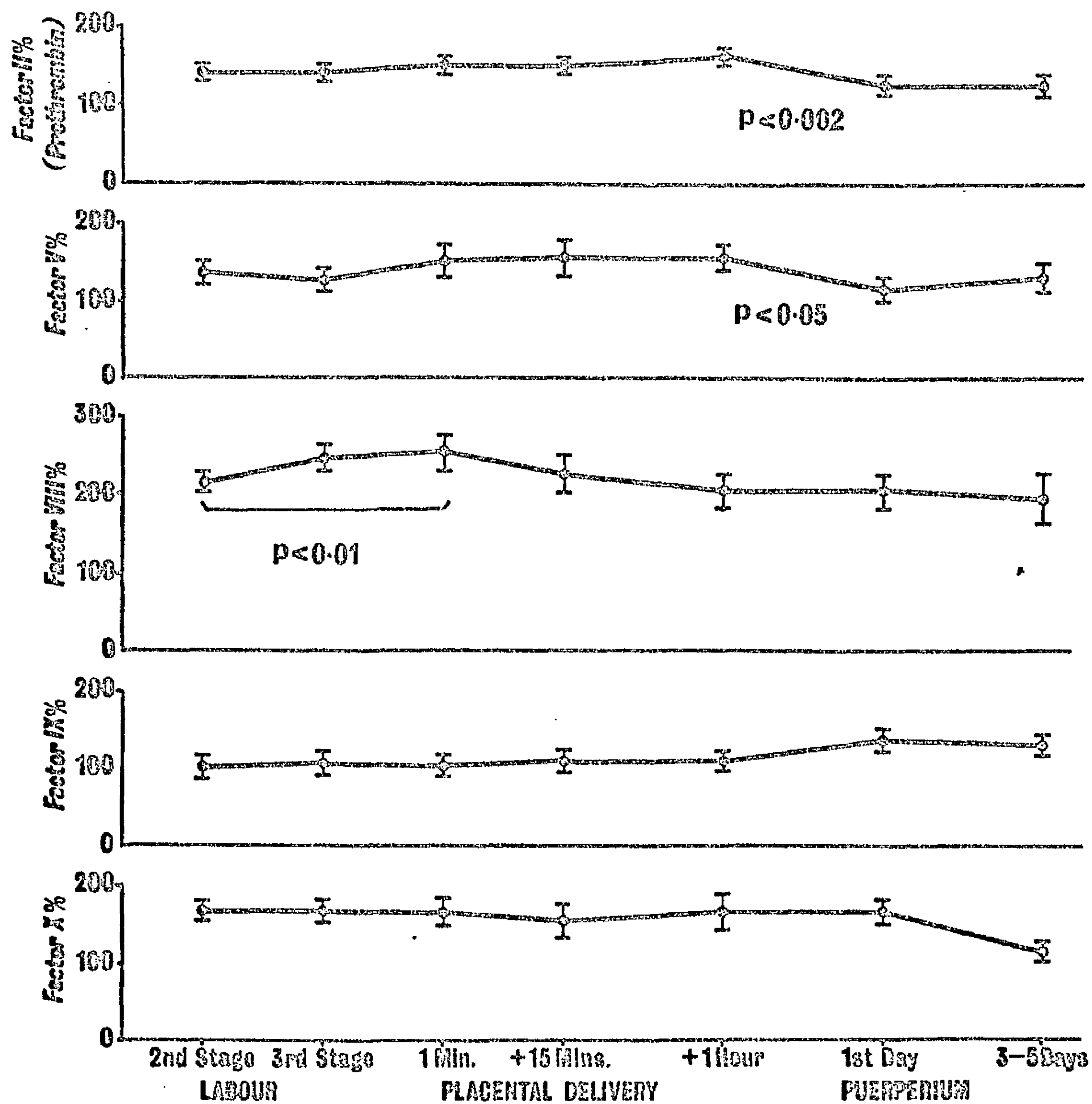

Figure 12

The effect of normal delivery on the activity of factors II, V, VIII, IX and X (mean values and standard error of the mean). The significant changes in the coagulation factors are shown. The figure is prepared from Tables 14, 15, 16, 17 and 18.

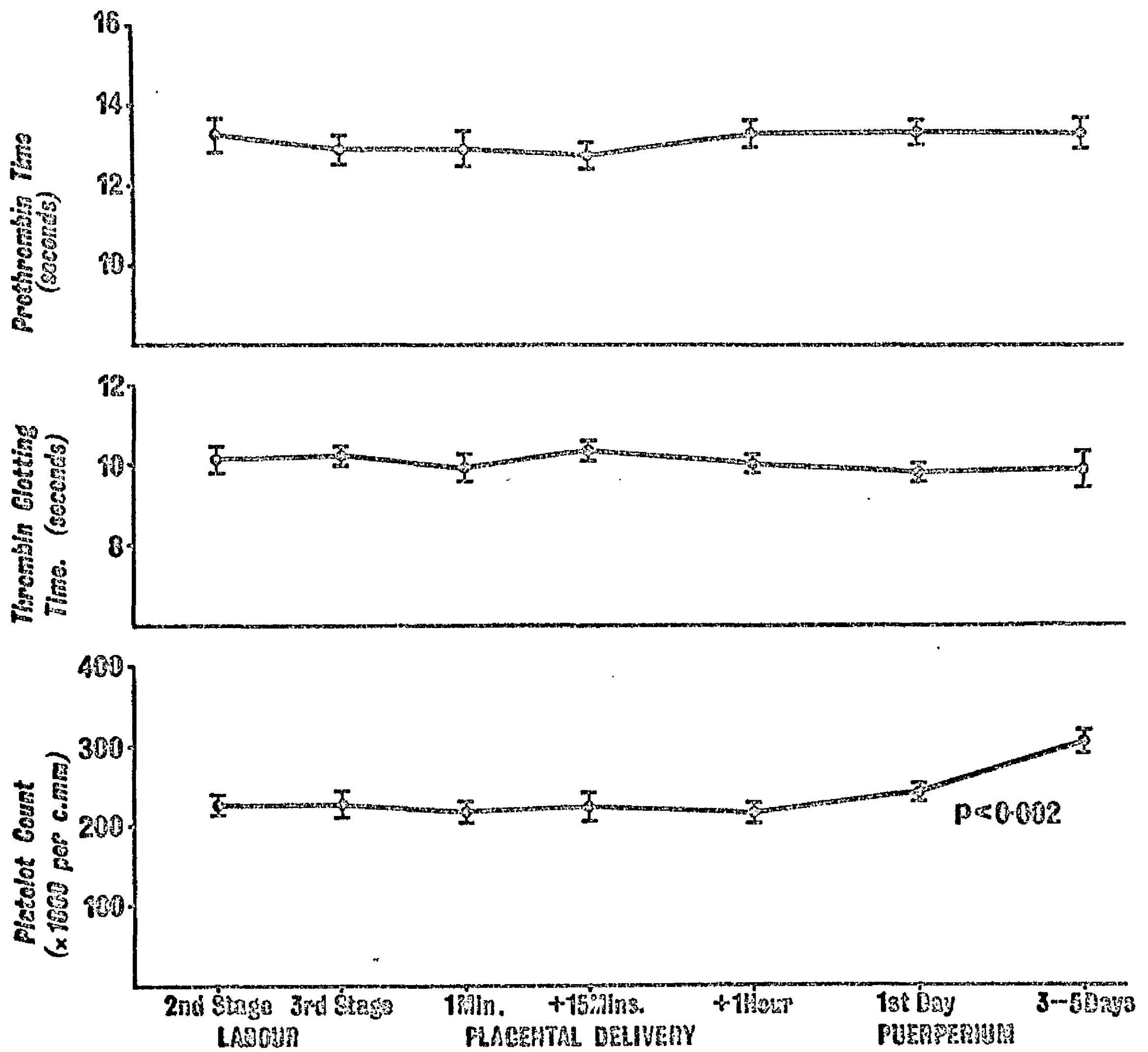

Figure 13

The effect of normal delivery on the prothrombin time, thrombin clotting time and platelet count (mean values and standard error of the mean). The sharp increase in the platelet count after delivery is shown. The figure is prepared from Tables 19, 20 and 21.

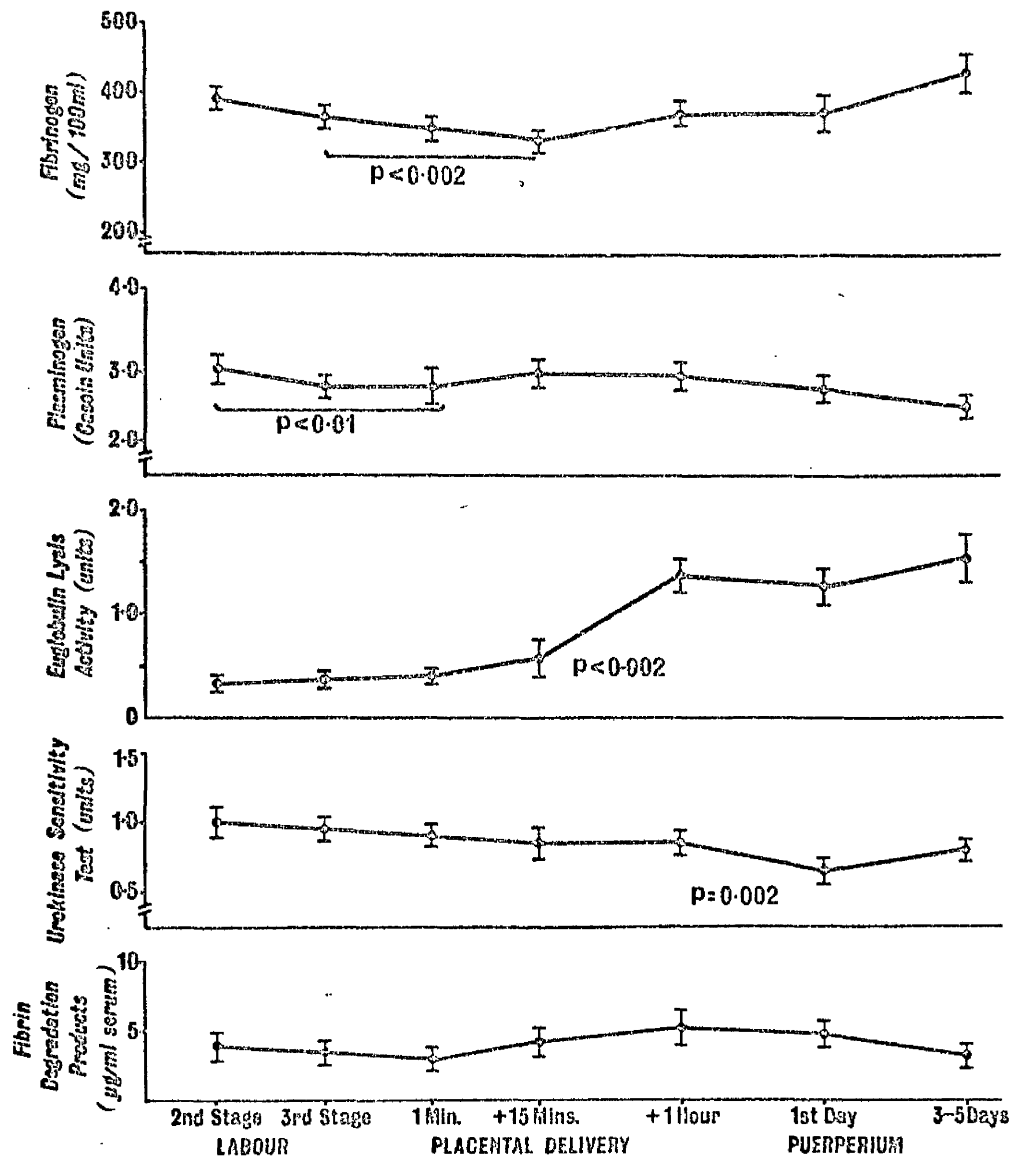

Figure 14

The effect of normal delivery on the fibrinolytic enzyme system. The changes in the levels of fibrinogen, plasminogen, euglobulin lysis activity, urokinase inhibitor and fibrin / fibrinogen degradation products are shown (mean values and standard error of the mean).
The figure is prepared from Tables 22, 23, 24, 25 and 26.

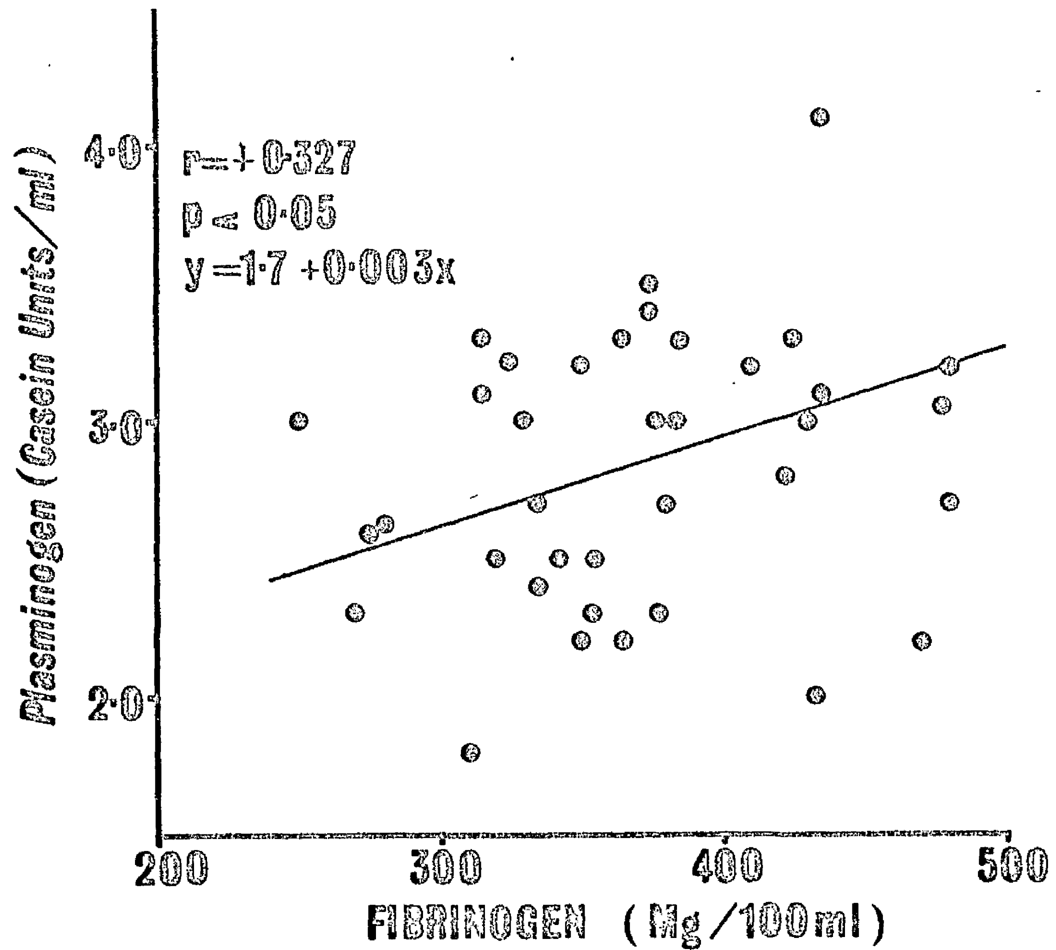

Figure 15

The correlation between the changes in plasma fibrinogen and plasma plasminogen during normal delivery.

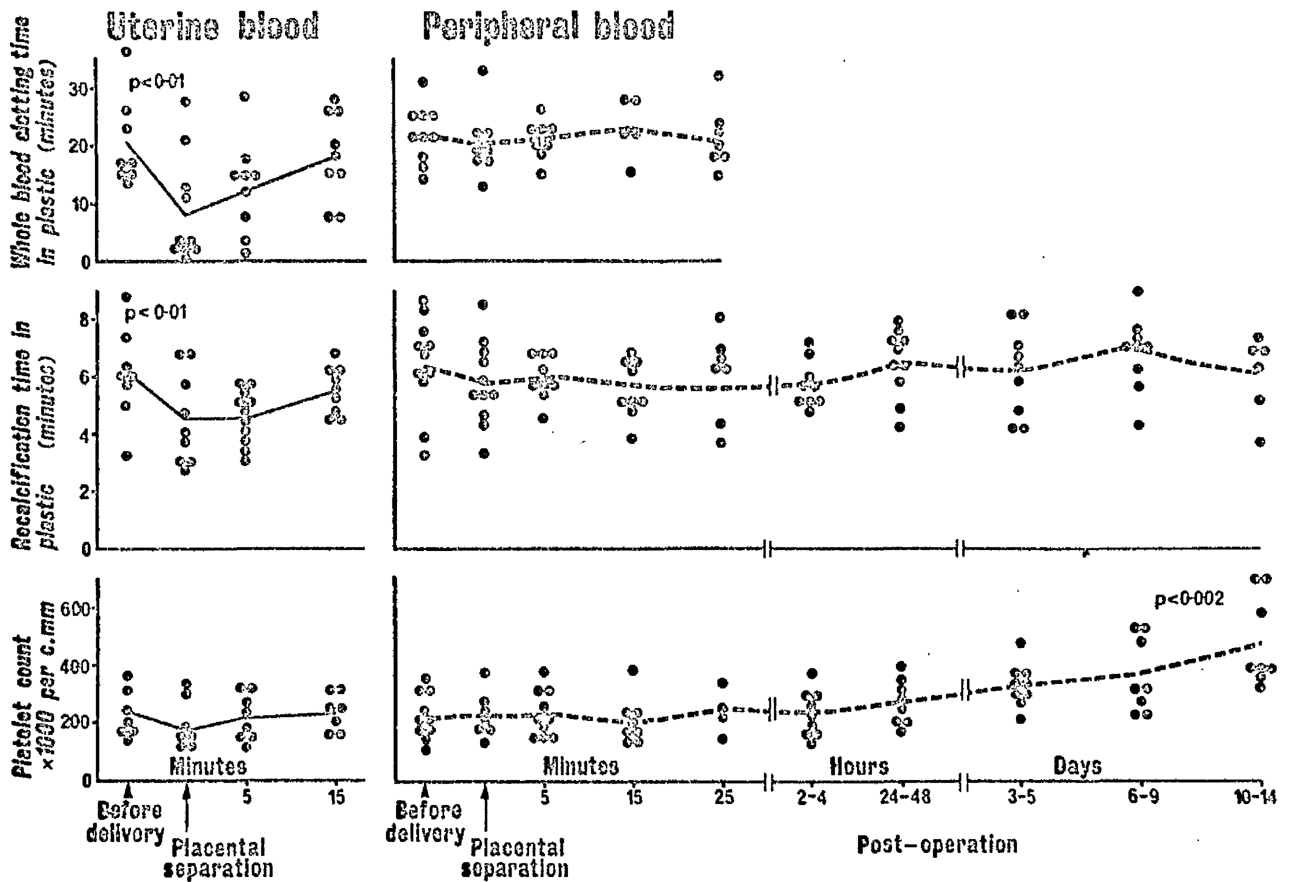

Figure 16

The whole blood clotting time in plastic, recalcification time and platelet count in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section. The figure is prepared from Tables 27, 28 and 29.

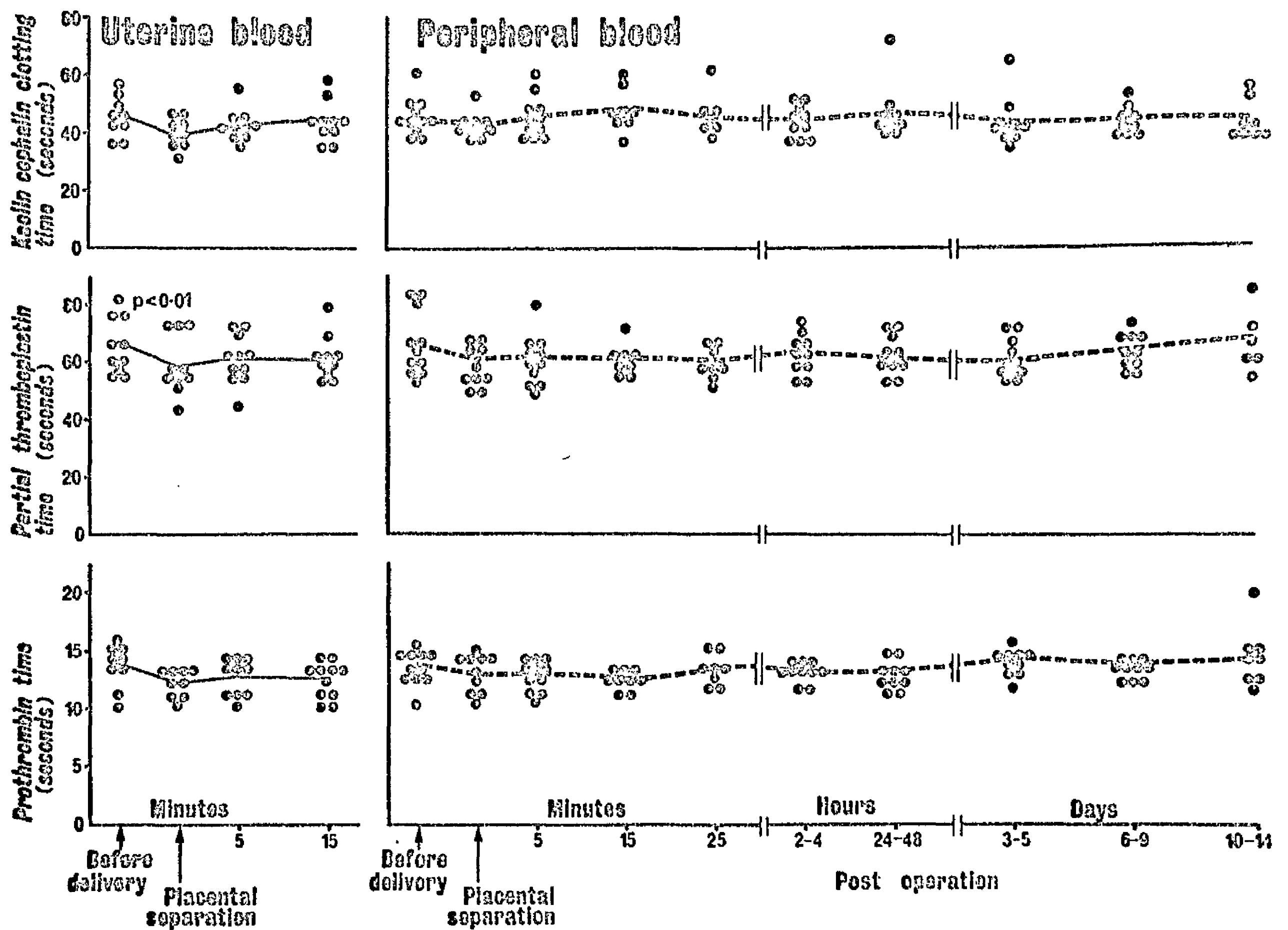

Figure 17

The kaolin-cephalin clotting time, partial thromboplastin time and prothrombin time in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section. The figure is prepared from Tables 30, 31 and 32.

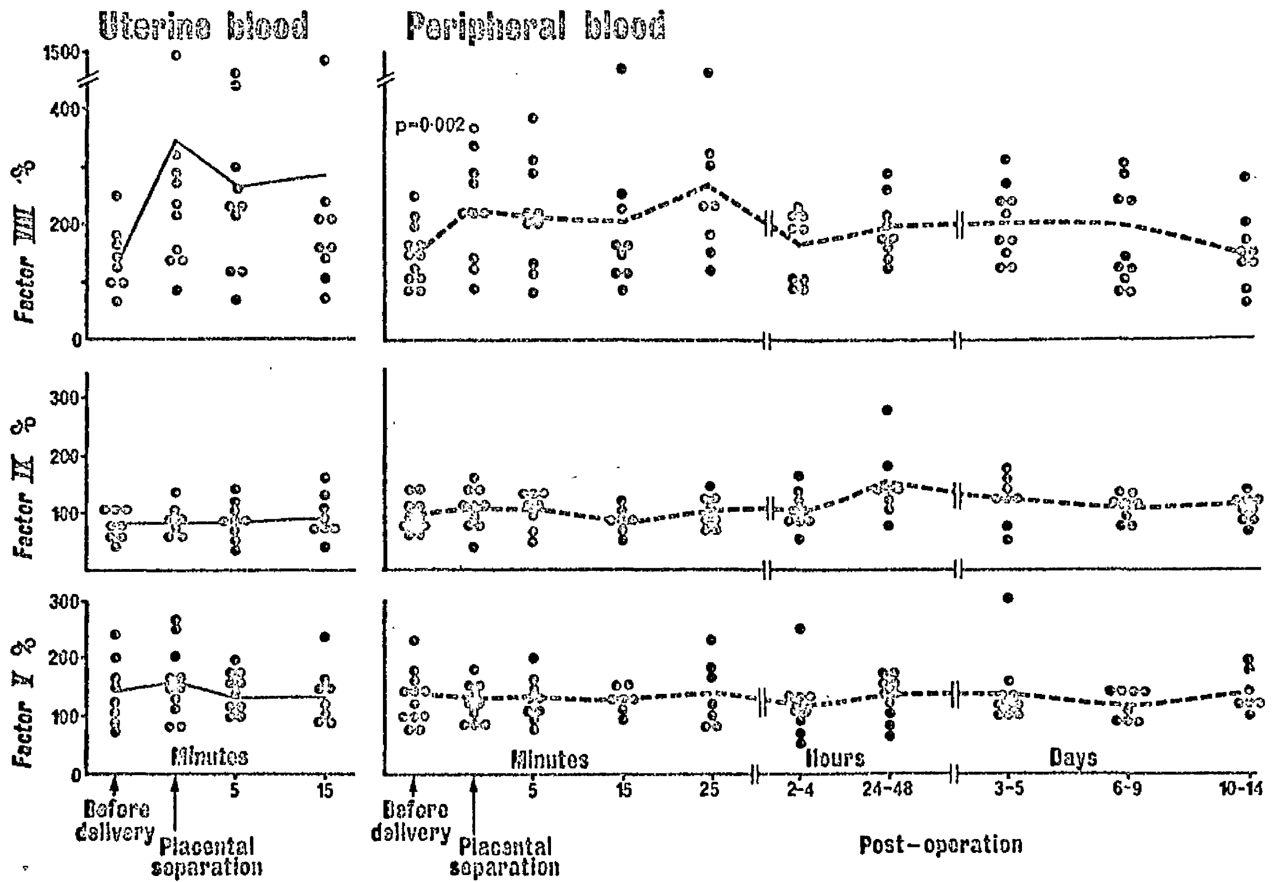

Figure 18

The levels of factors VIII, IX and V in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section. The figure is prepared from Tables 34, 35 and 36.

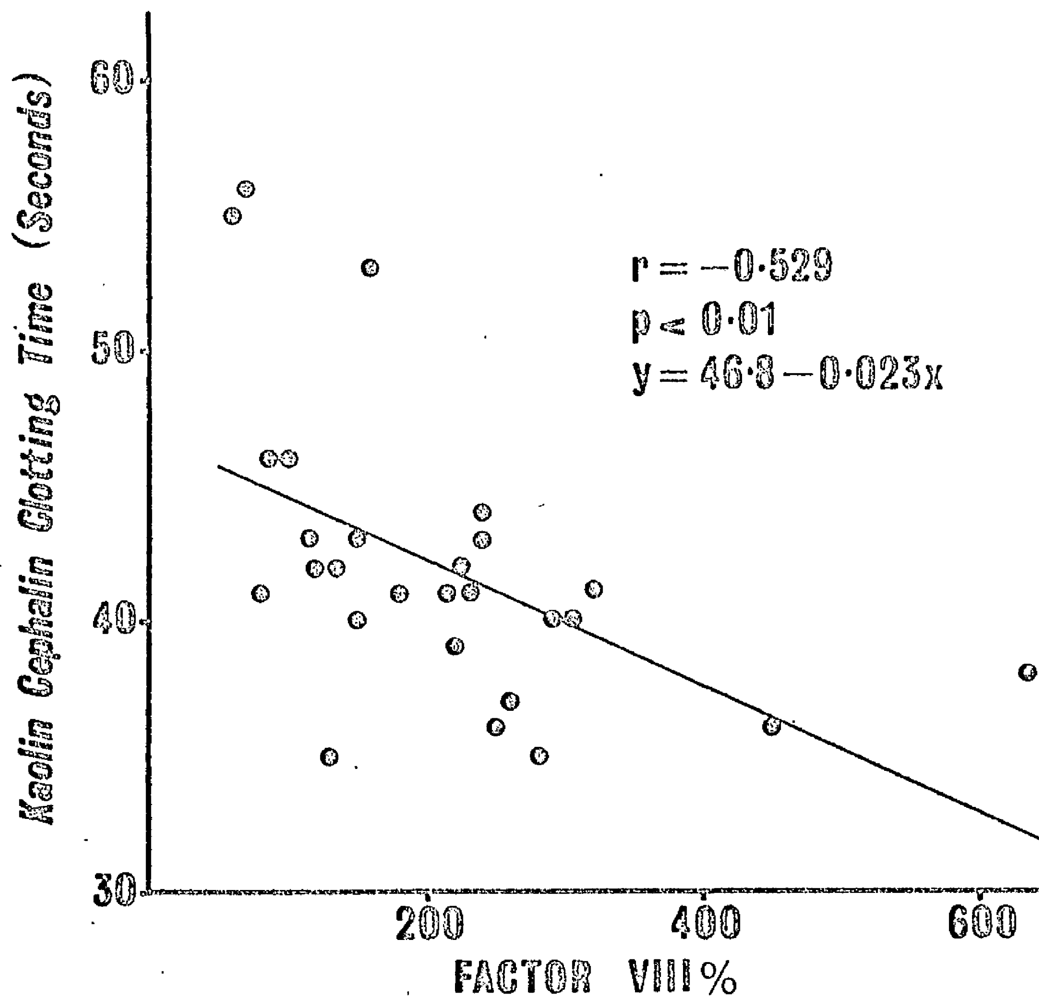

Figure 19

The correlation between the changes in factor VIII activity and the kaolin-cephalin clotting time in uterine vein blood during and after placental separation.

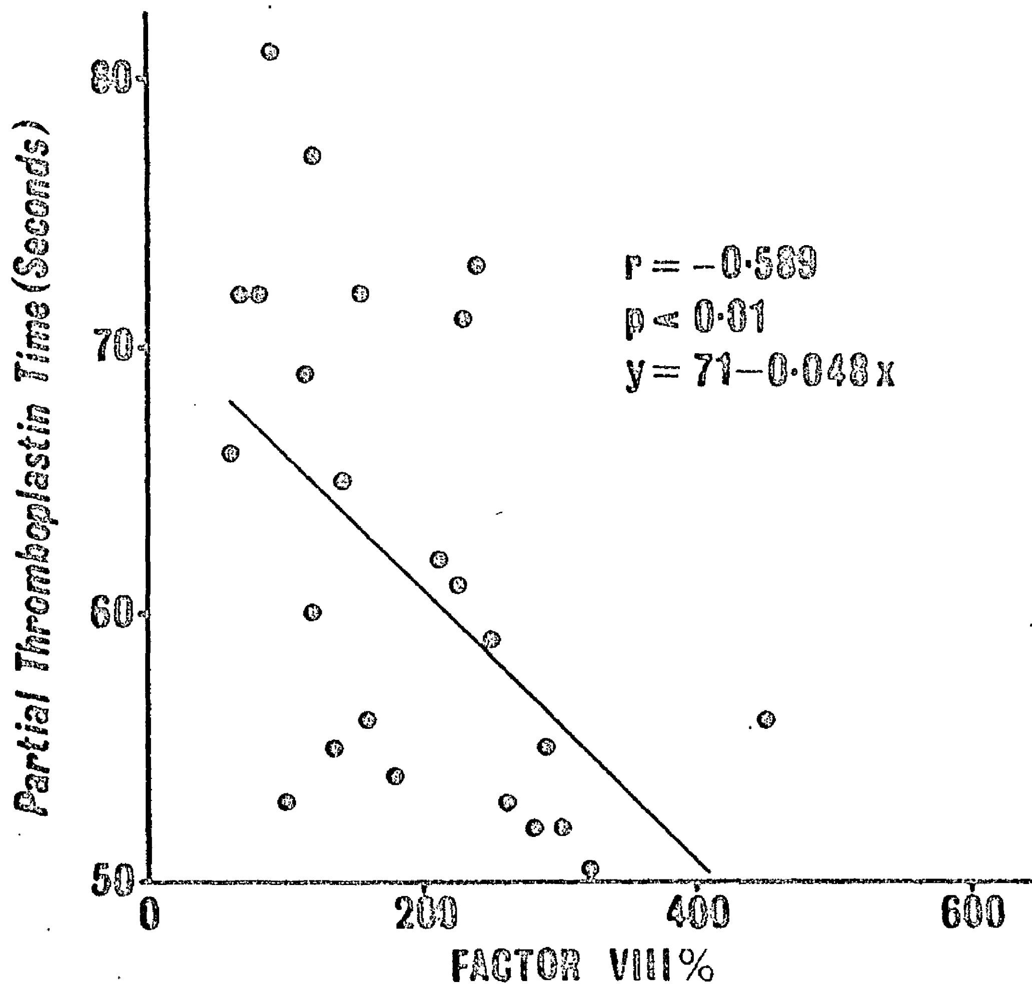

Figure 20

The correlation between the changes in factor VIII activity and the partial thromboplastin time in uterine vein blood during and after placental separation.

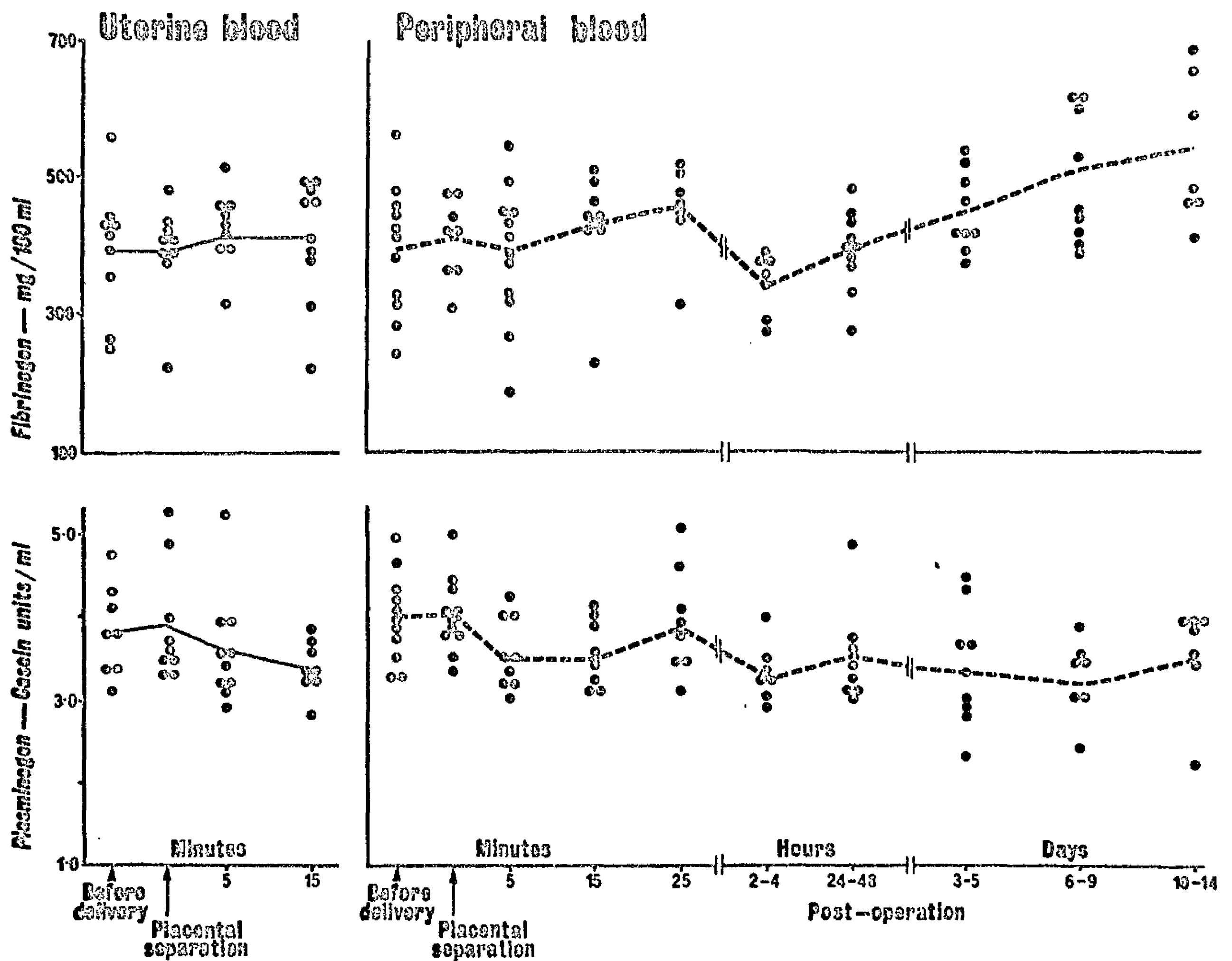

Figure 21

The plasma fibrinogen and plasminogen levels in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section. The figure is prepared from Tables 37 and 38.

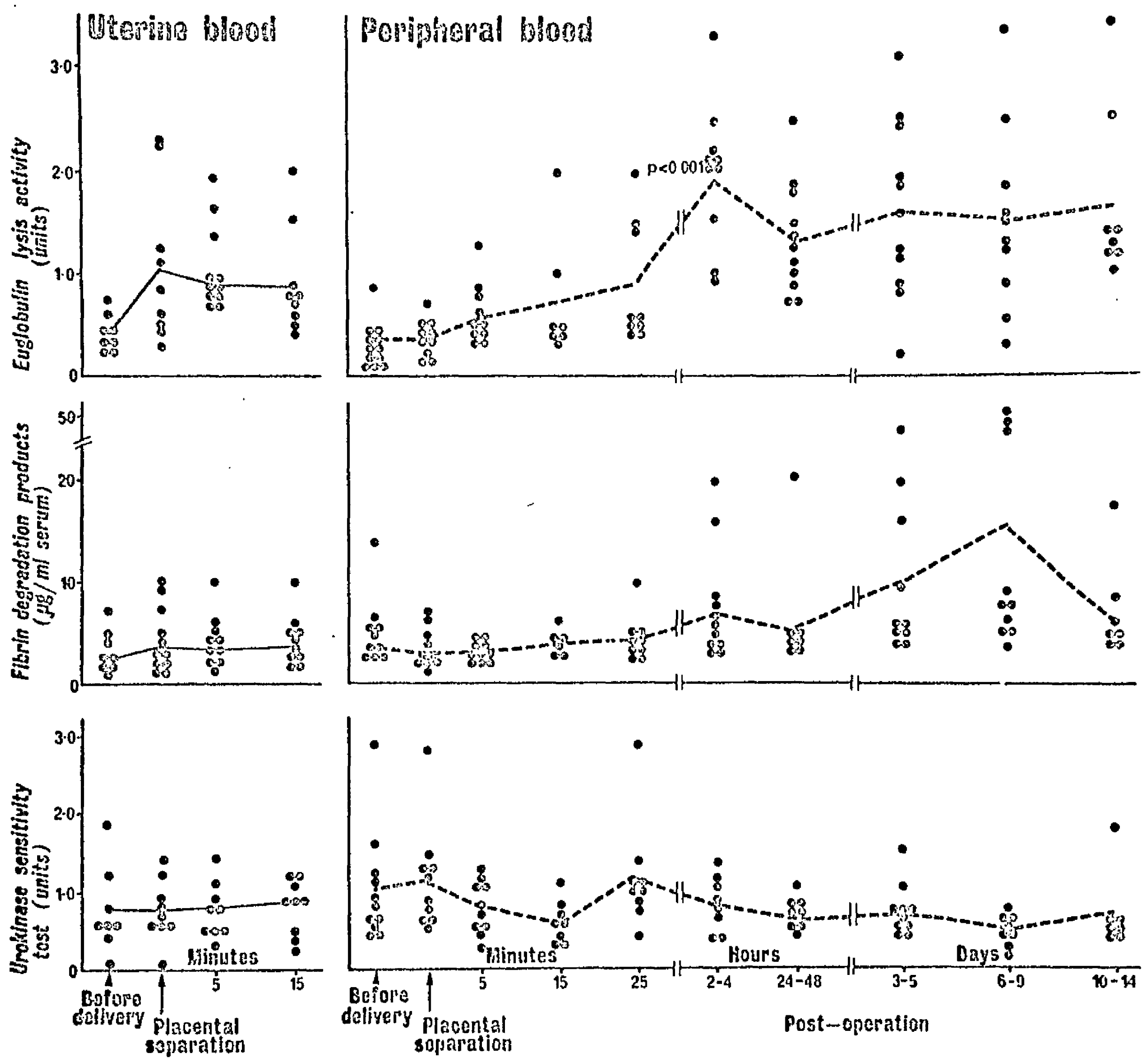

Figure 22

Euglobulin lysis activity, fibrin / fibrinogen degradation products in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section. The figure is prepared from Tables 39, 40 and 41.

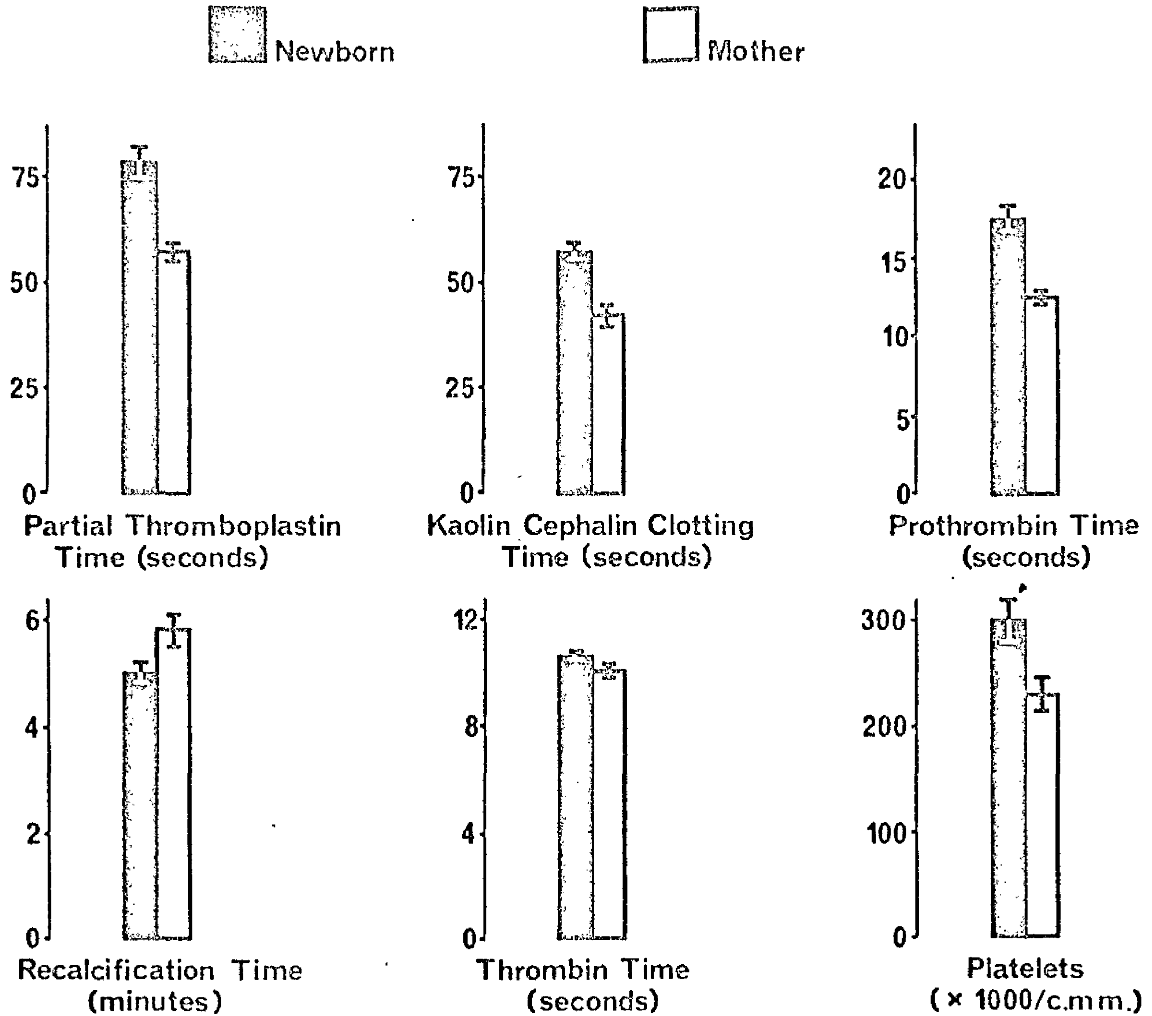

Figure 23

The clotting tests in the maternal blood and cord blood immediately after birth (mean + standard error). With the exception of the recalcification time, the clotting tests in the baby were significantly longer than the maternal values. The figure is prepared from Table 42 and the platelet counts from Table 43A.

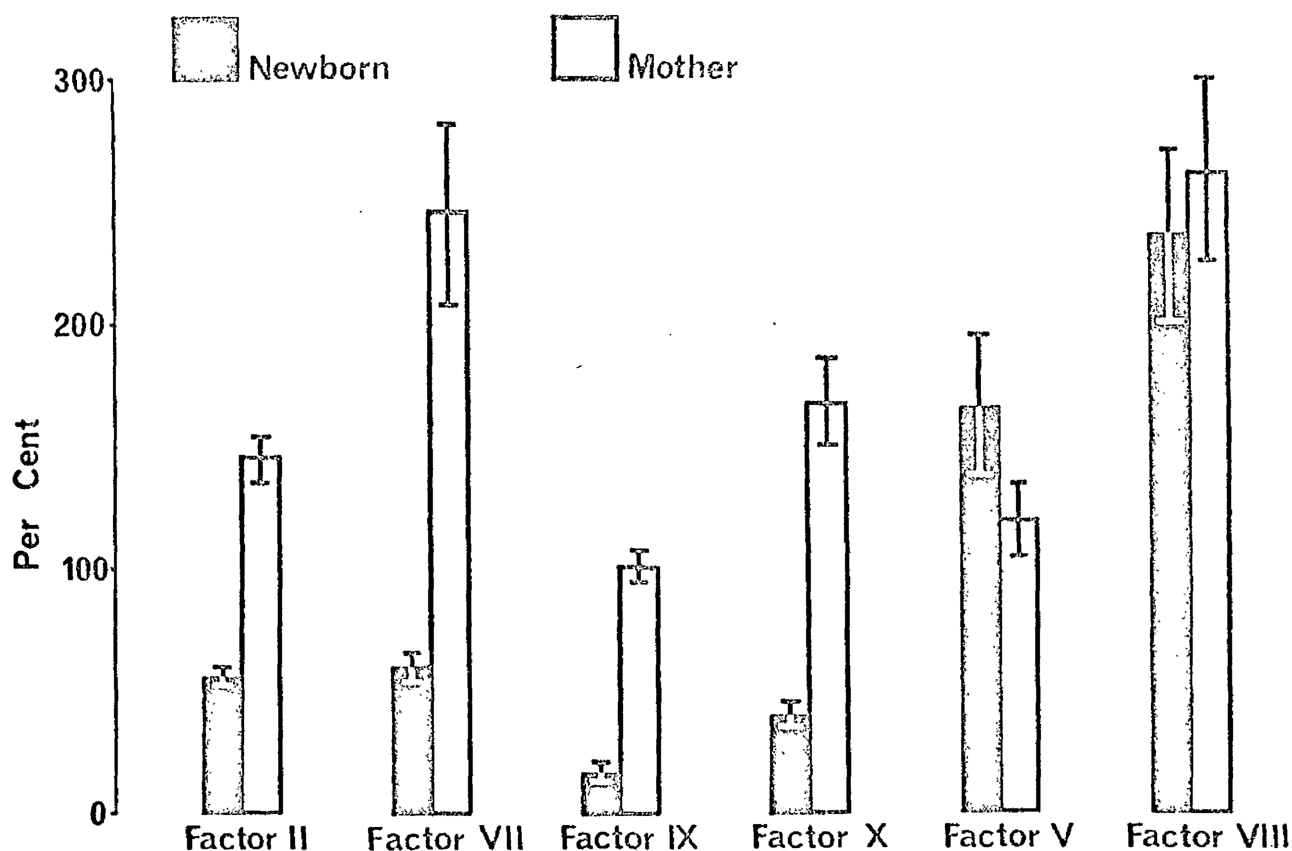

Figure 24

The coagulation factors in the maternal blood and cord blood immediately after birth (mean + standard error). Factors II (prothrombin), VII, IX, and X in the baby were markedly lower than in the mother but no significant difference was present between factors V and VIII in the baby and the mother. The figure is prepared from Tables 43 A and 43 B.

THE FIBRINOLYTIC ENZYME SYSTEM IN THE NEWBORN AND IN THE MOTHER

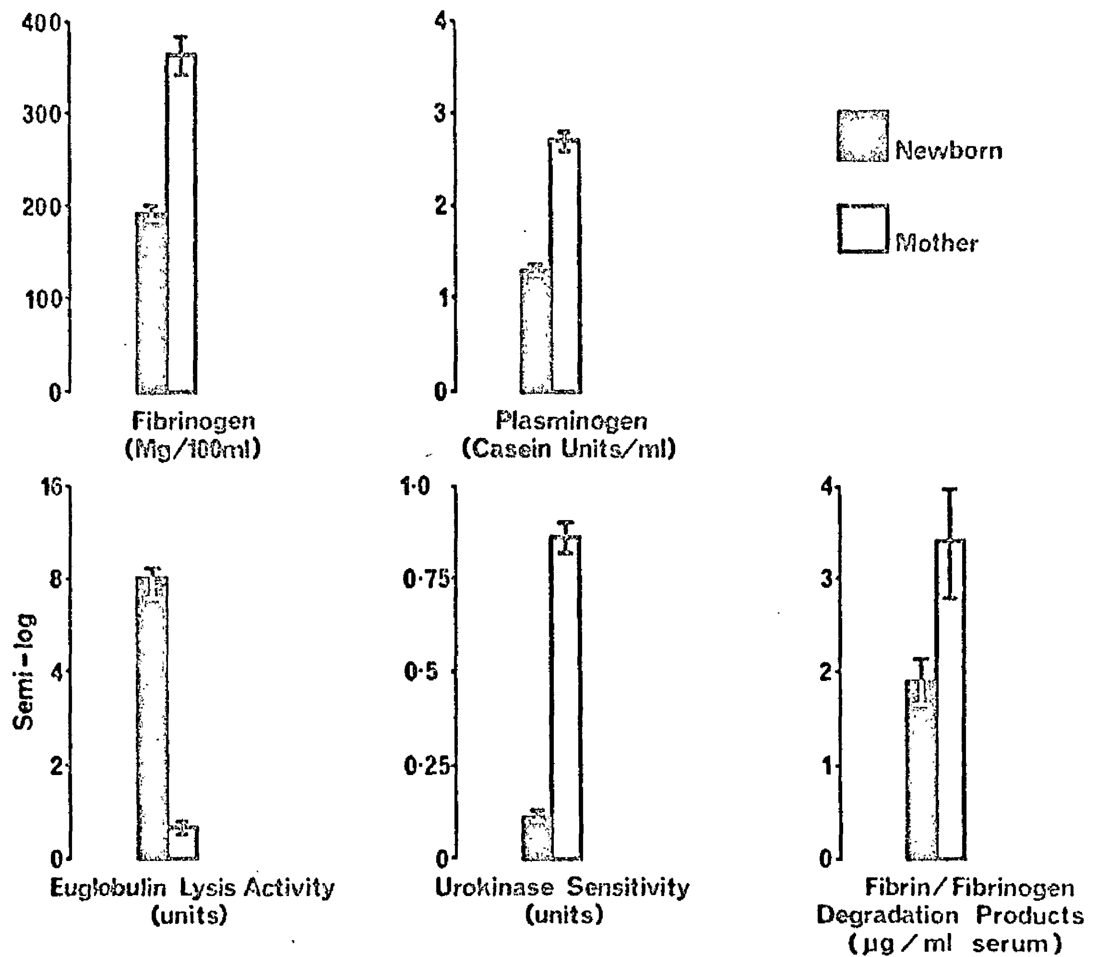

Figure 25

The components of the fibrinolytic enzyme system in the maternal blood and cord blood immediately after birth (mean + standard error). The high level of fibrinolytic activity and reduced sensitivity to urokinase in the baby contrasted sharply with the maternal values. The figure is prepared from Table 44.

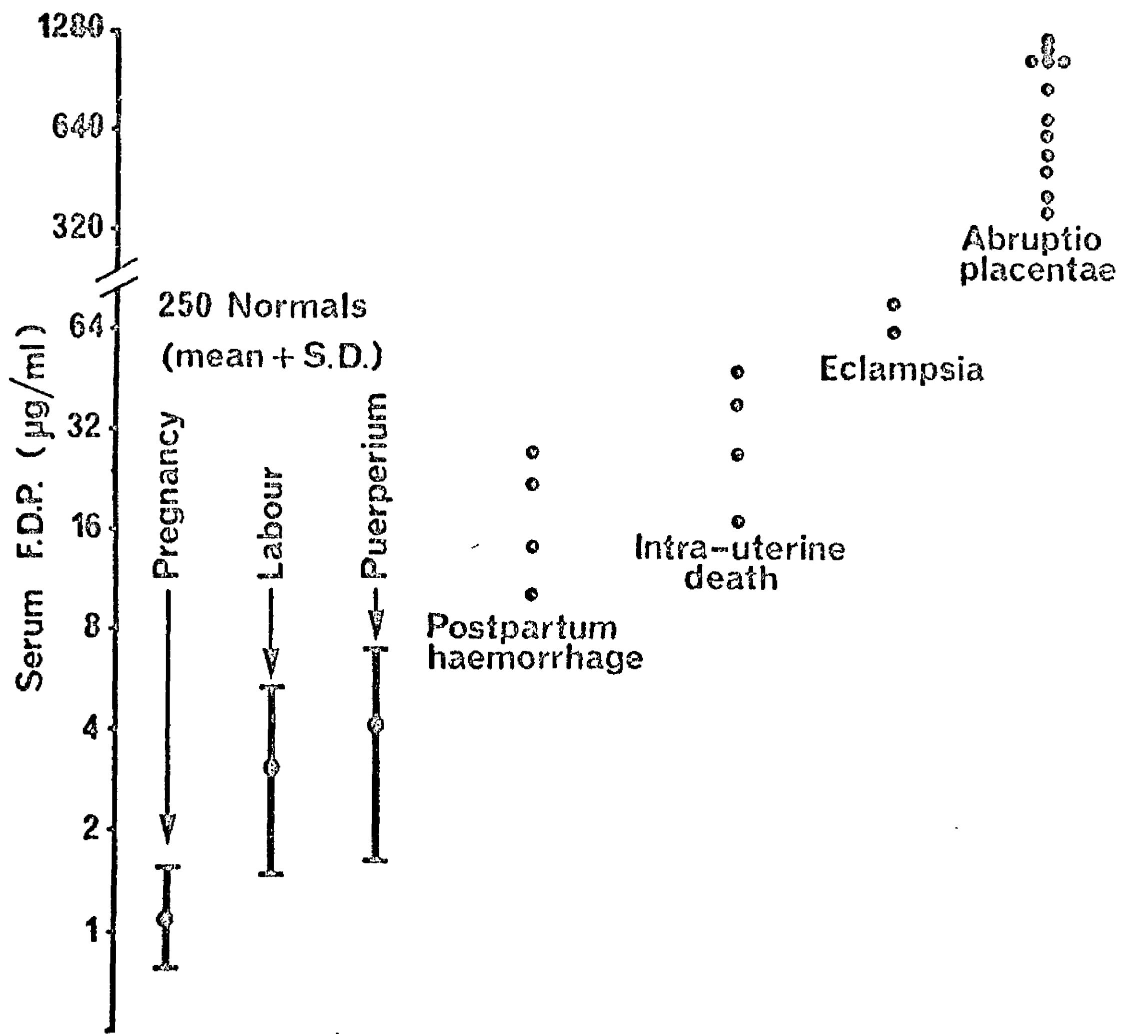

Figure 26

Levels of fibrin/fibrinogen degradation products (F.D.P.) in the serum of patients with abruptio placentae, eclampsia, intrauterine death, and after post-partum haemorrhage, compared with mean levels found in 250 healthy women in normal pregnancy, labour and the puerperium (logarithmic scale).

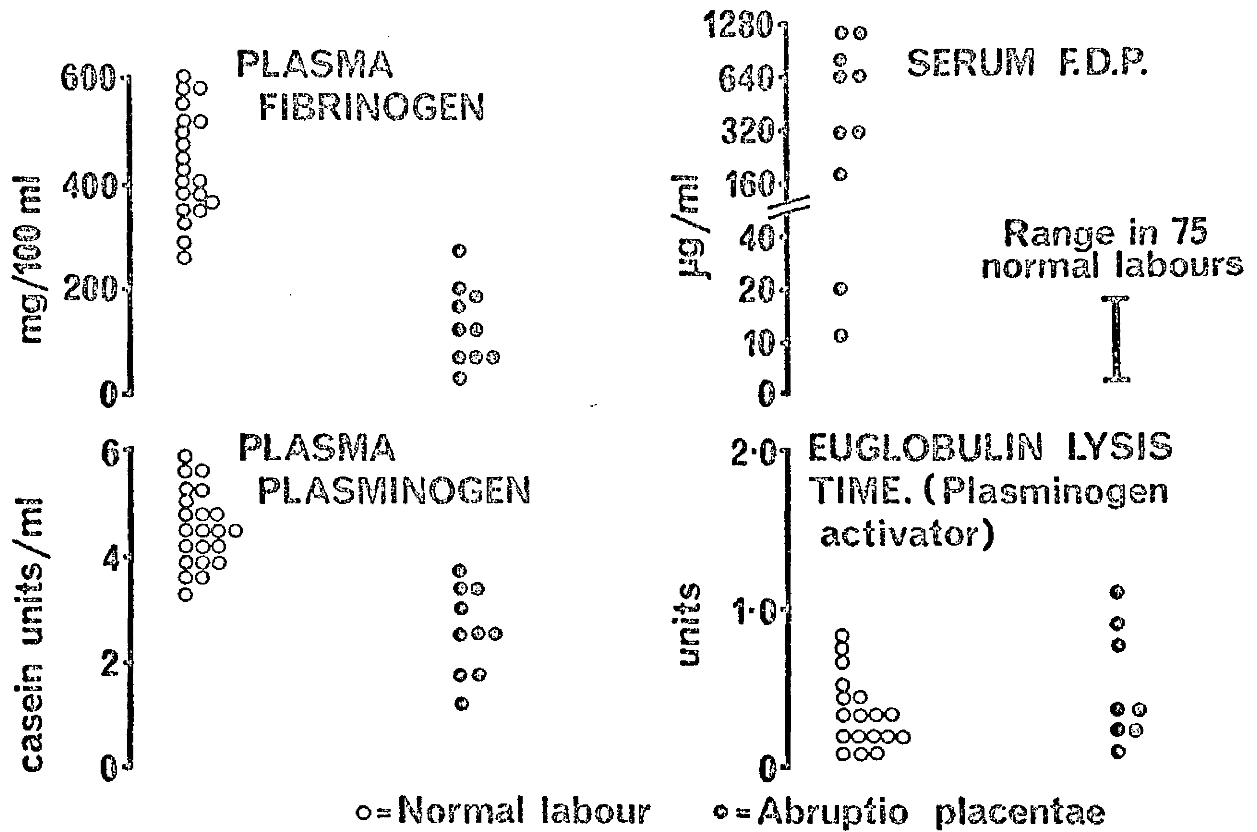

Figure 27

The levels of fibrinogen, plasminogen, serum fibrin / fibrinogen degradation products (F.D.P.) and plasminogen activator in 10 patients with abruptio placentae prior to treatment, as compared with the findings in 20 healthy patients in normal labour.

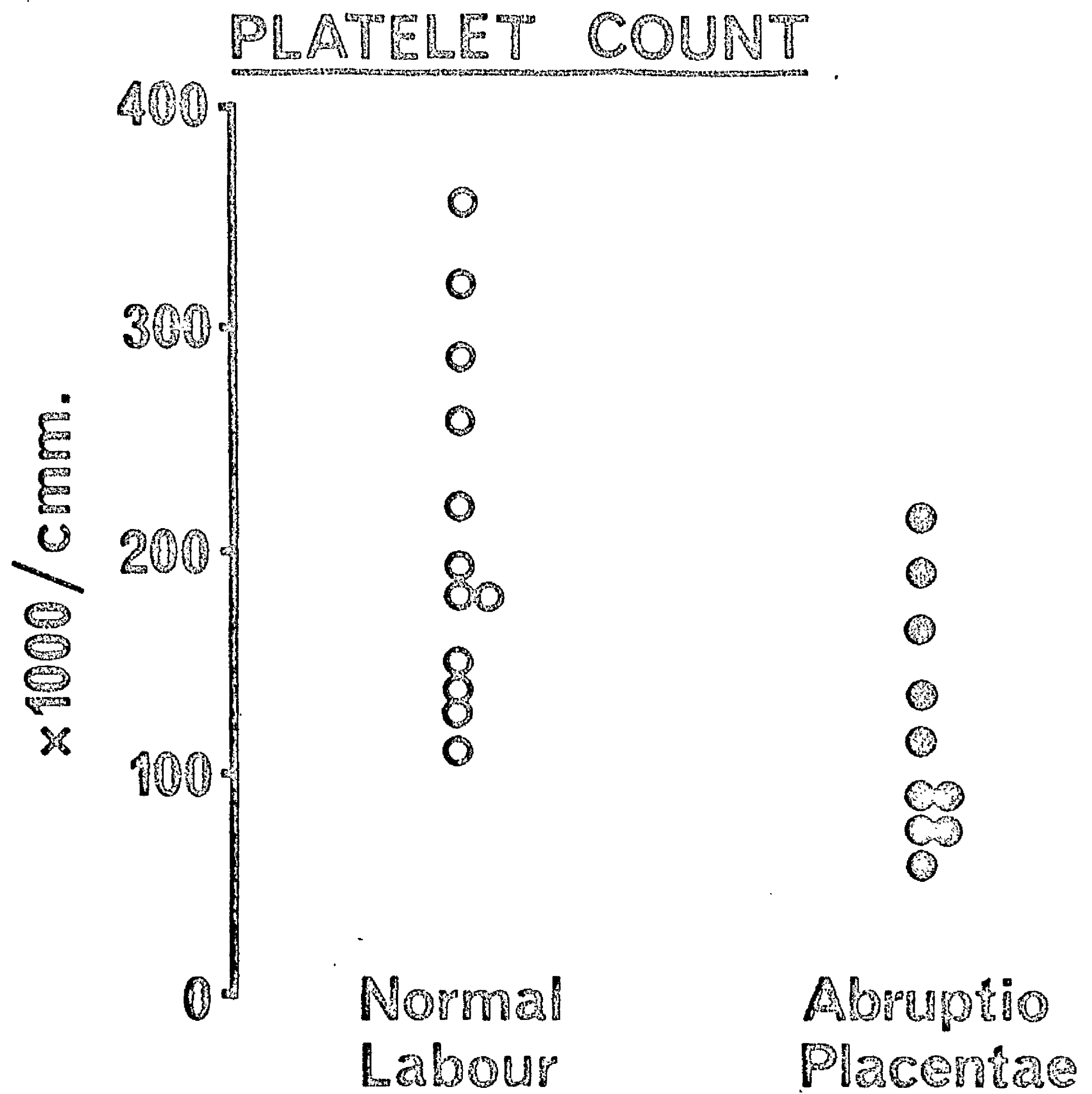

Figure 28

The platelet counts in the same patients as in figure 27 before blood transfusion was commenced.

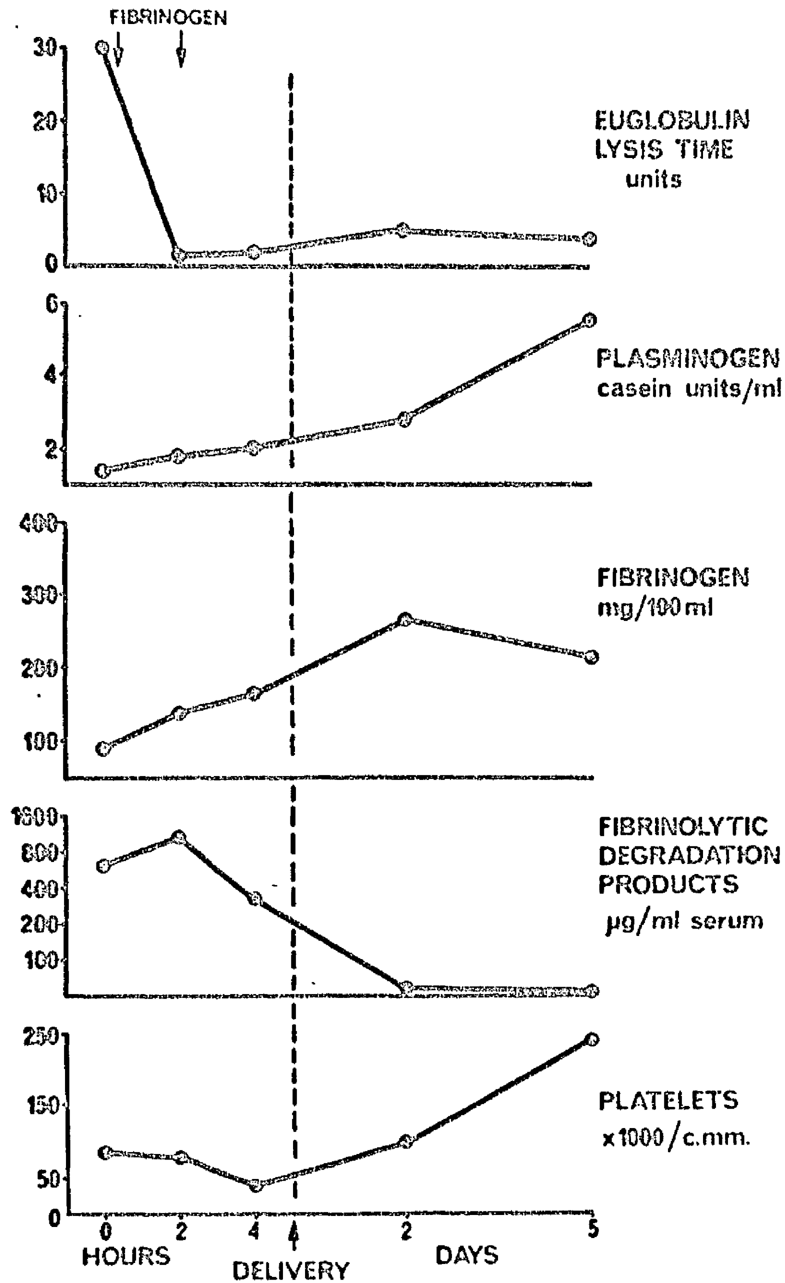

Figure 29

The findings in a patient with severe abruptio placentae where transient evidence of a systemic proteolytic state was present as shown by the high level of plasminogen activator (euglobulin lysis time). Fibrinogen (4 g) was given and the fibrin degradation products increased from 600 µg /ml to 1200 µg /ml.

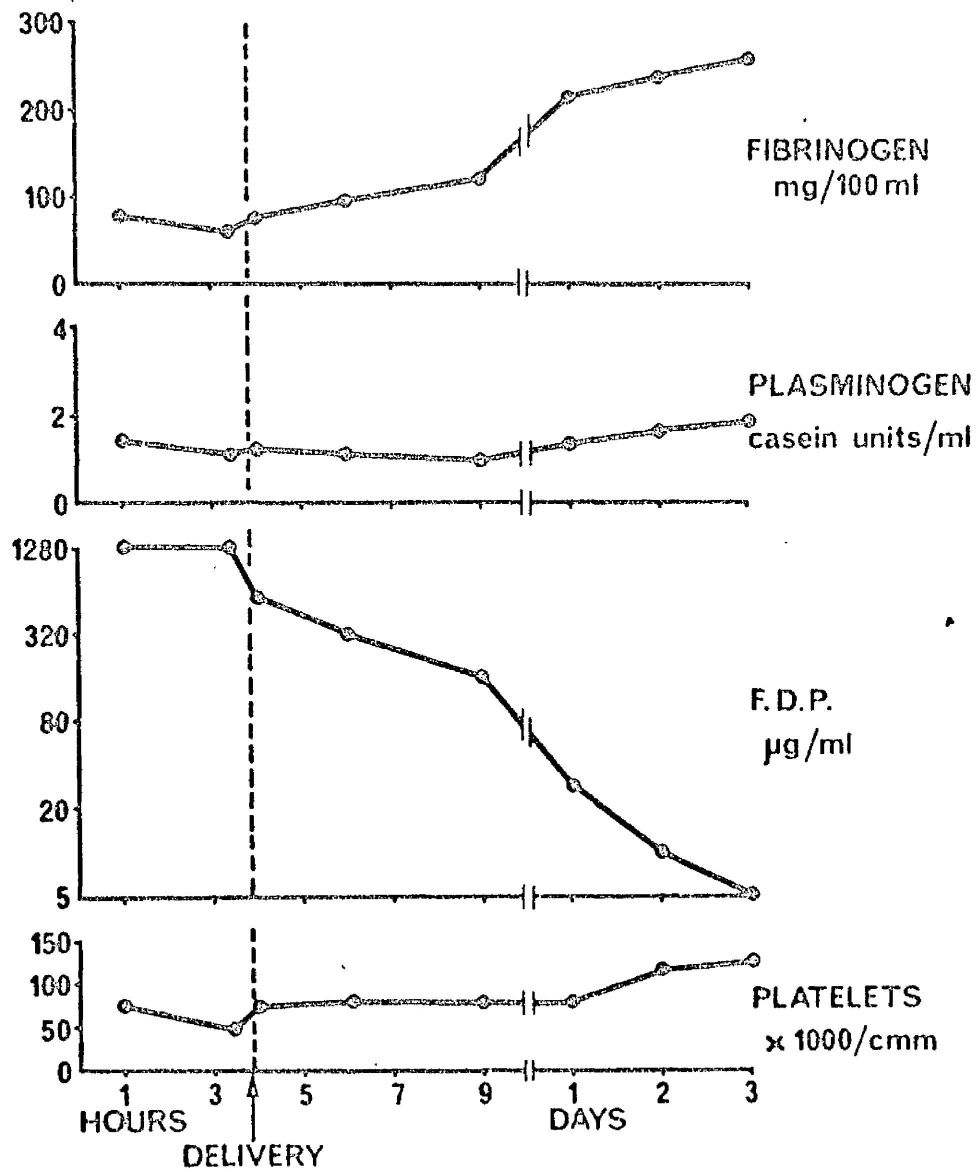

Figure 30

Levels of fibrinogen, plasminogen, fibrin degradation products (F.D.P.) and platelets in severe abruptio placentae before and after delivery.

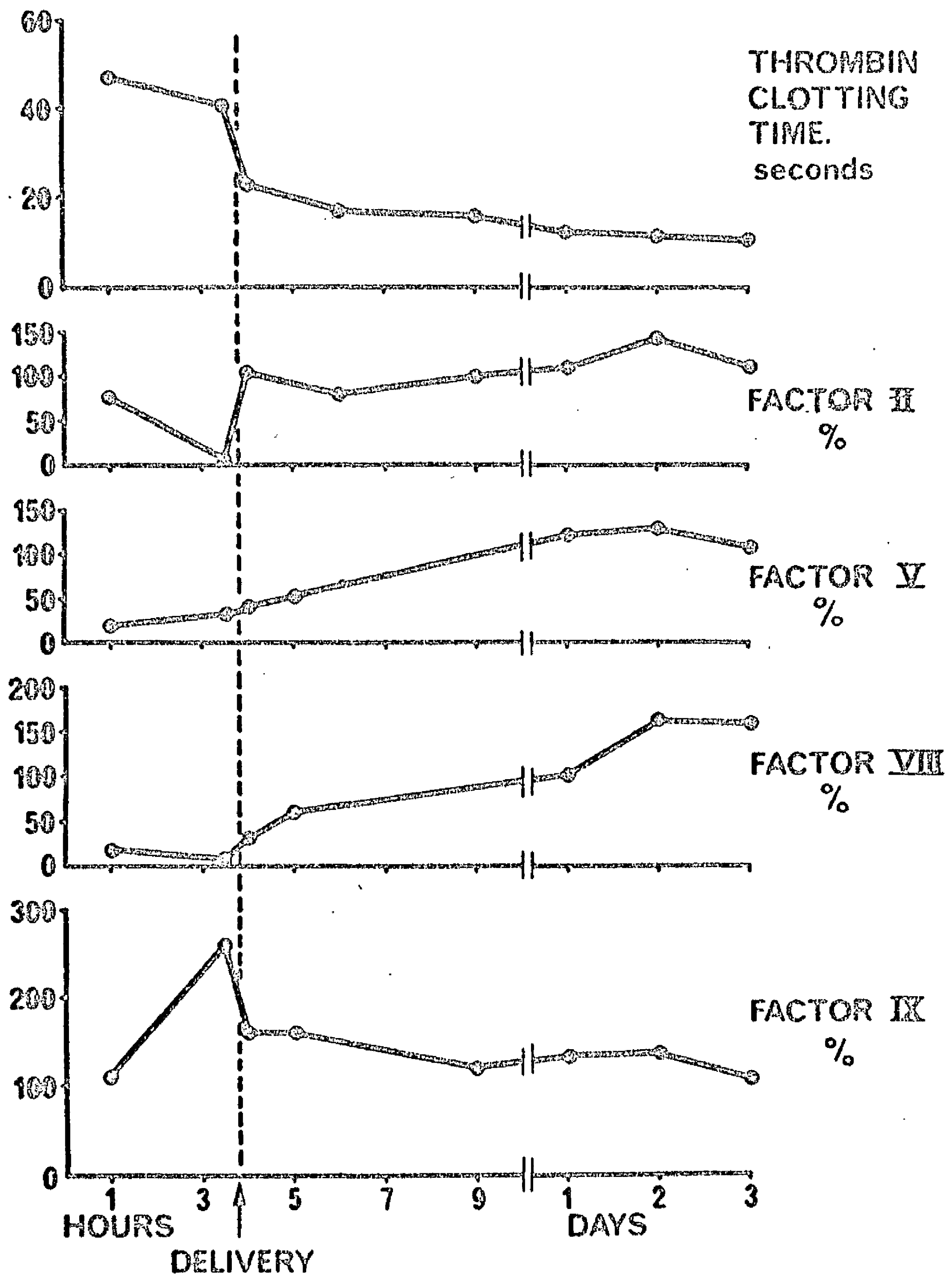

Figure 31

Thrombin clotting time, factor II, V, VIII and IX before and after delivery in the same patient as in figure 30.

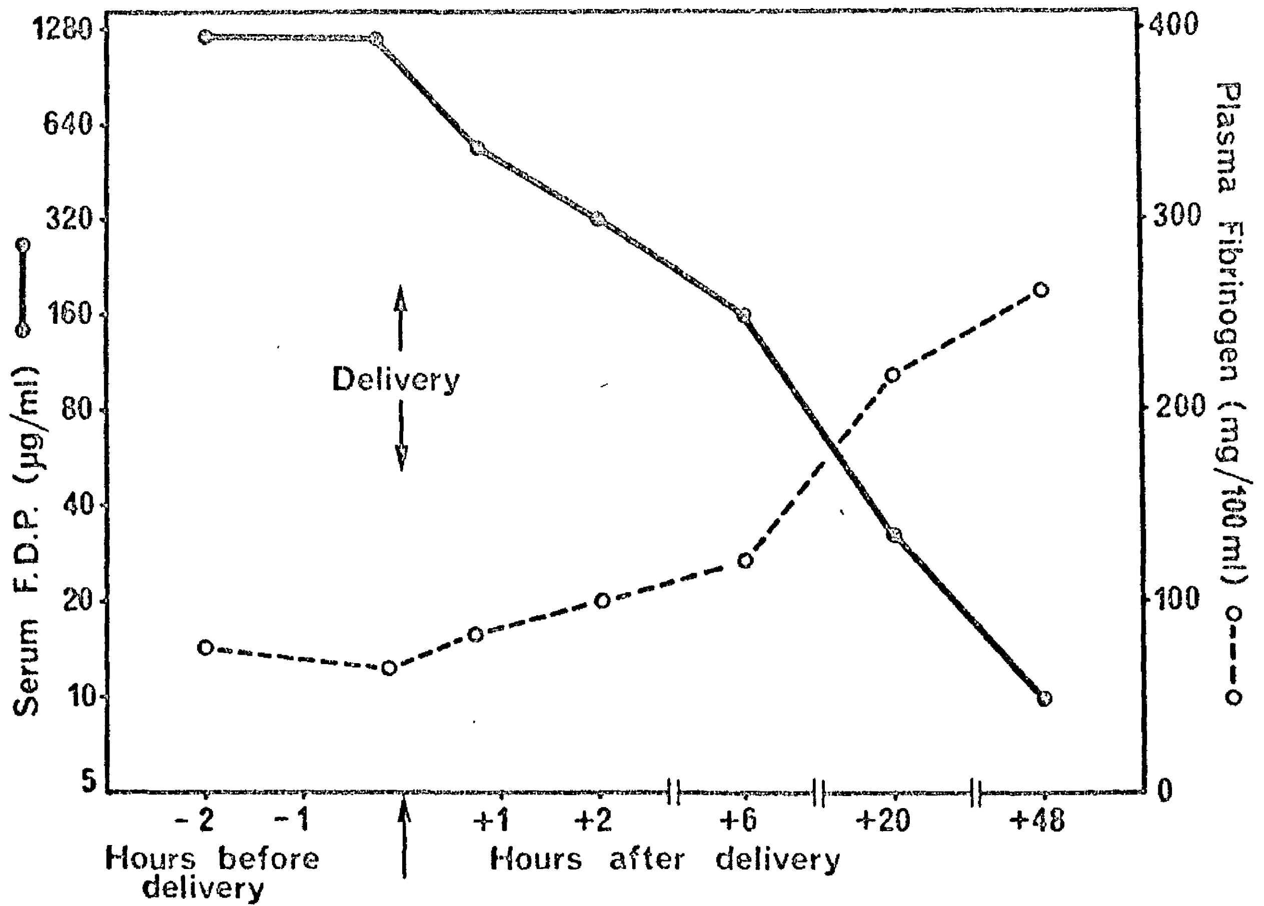

Figure 32

Spontaneous decrease of serum fibrin / fibrinogen degradation products (F.D.P.), (semi-log. scale) and increase of plasma fibrinogen after vaginal delivery in a patient with abruptio placentae.

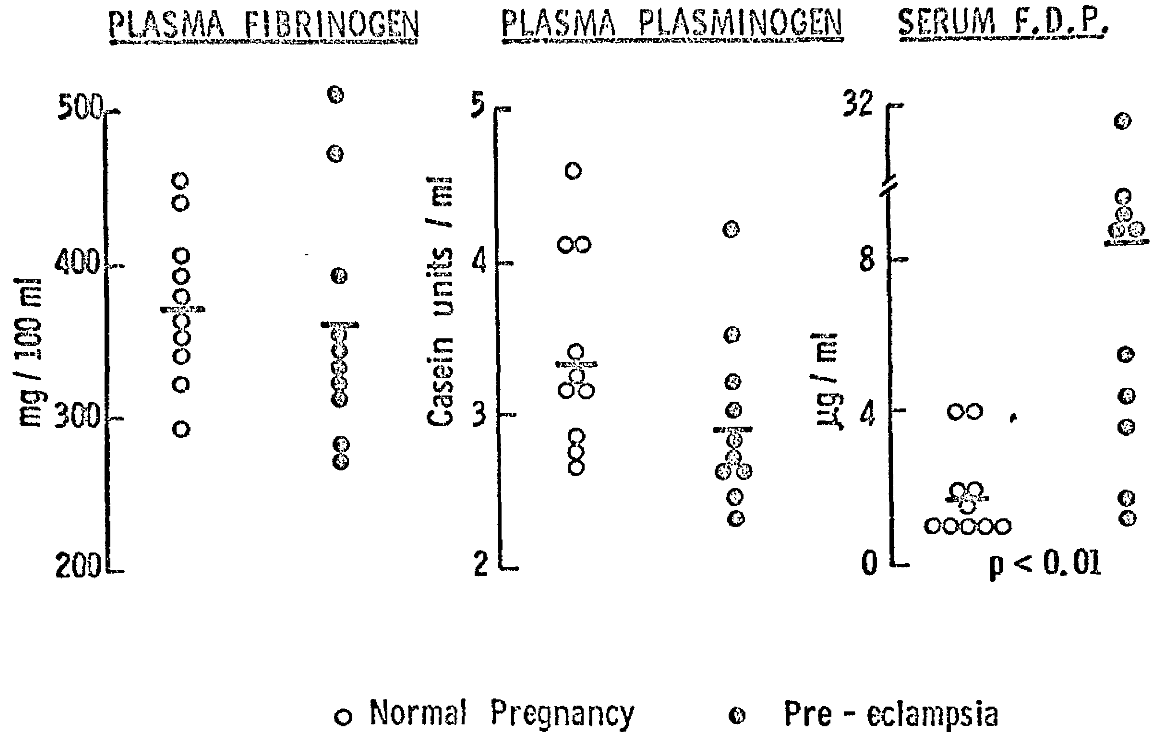

Figure 33

The levels and mean values of fibrinogen, plasminogen, and serum fibrin degradation products (F.D.P.) in 10 patients with severe pre-eclampsia as compared with the findings in 10 healthy patients matched for age and gestation. The figure is prepared from Table 46.

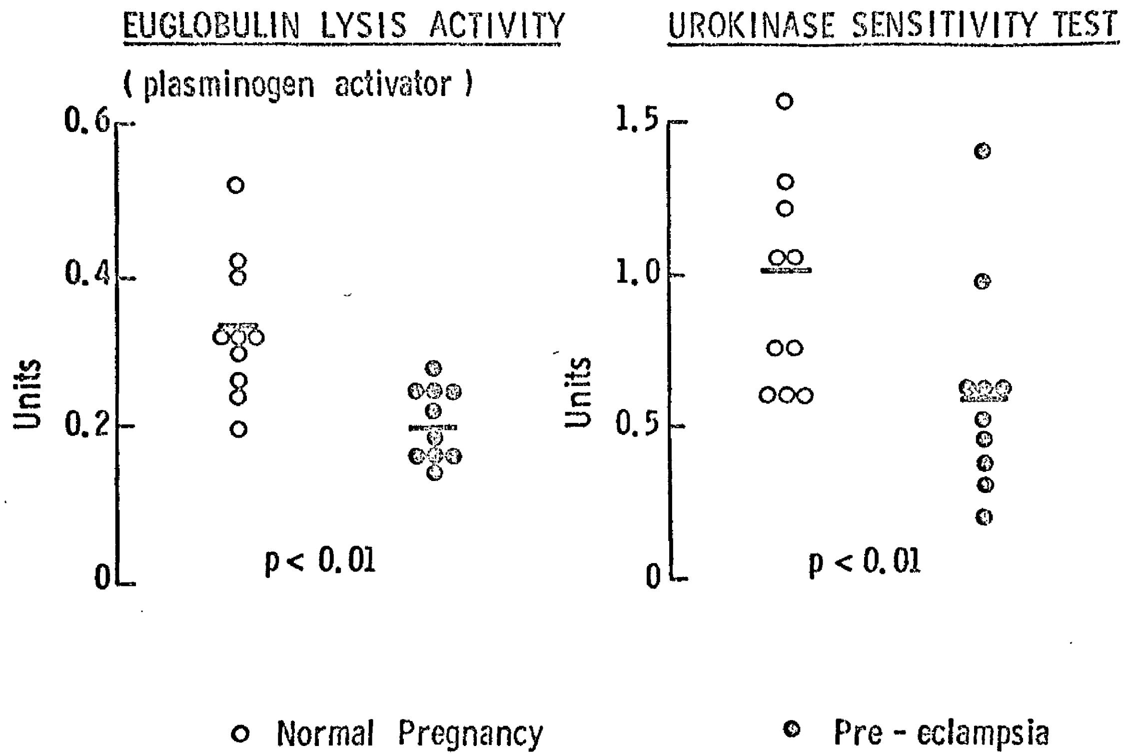

Figure 34

Euglobulin lysis activity and urokinase sensitivity results with mean values in severe pre-eclampsia compared with the control patients. The figure is prepared from Table 47.

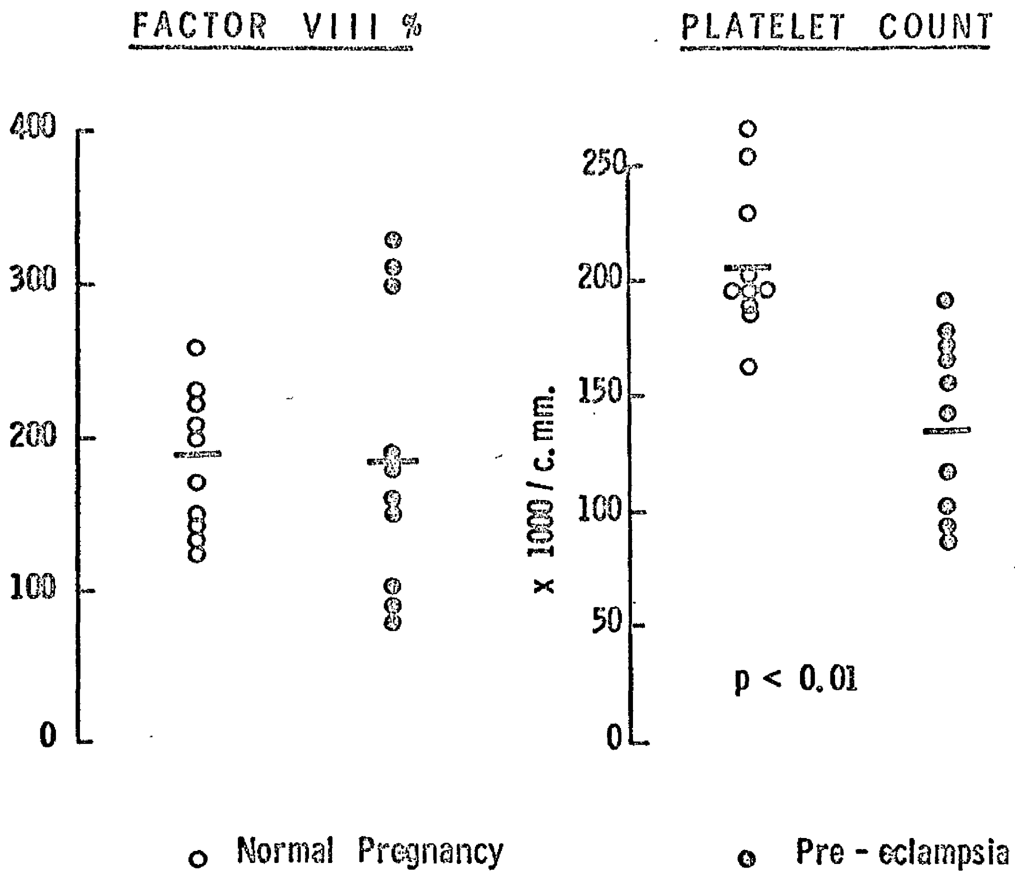

Figure 35

The levels and mean values of factor VIII and the platelet count in patients with severe pre-eclampsia compared with the control patients. The figure is prepared from Table 48.

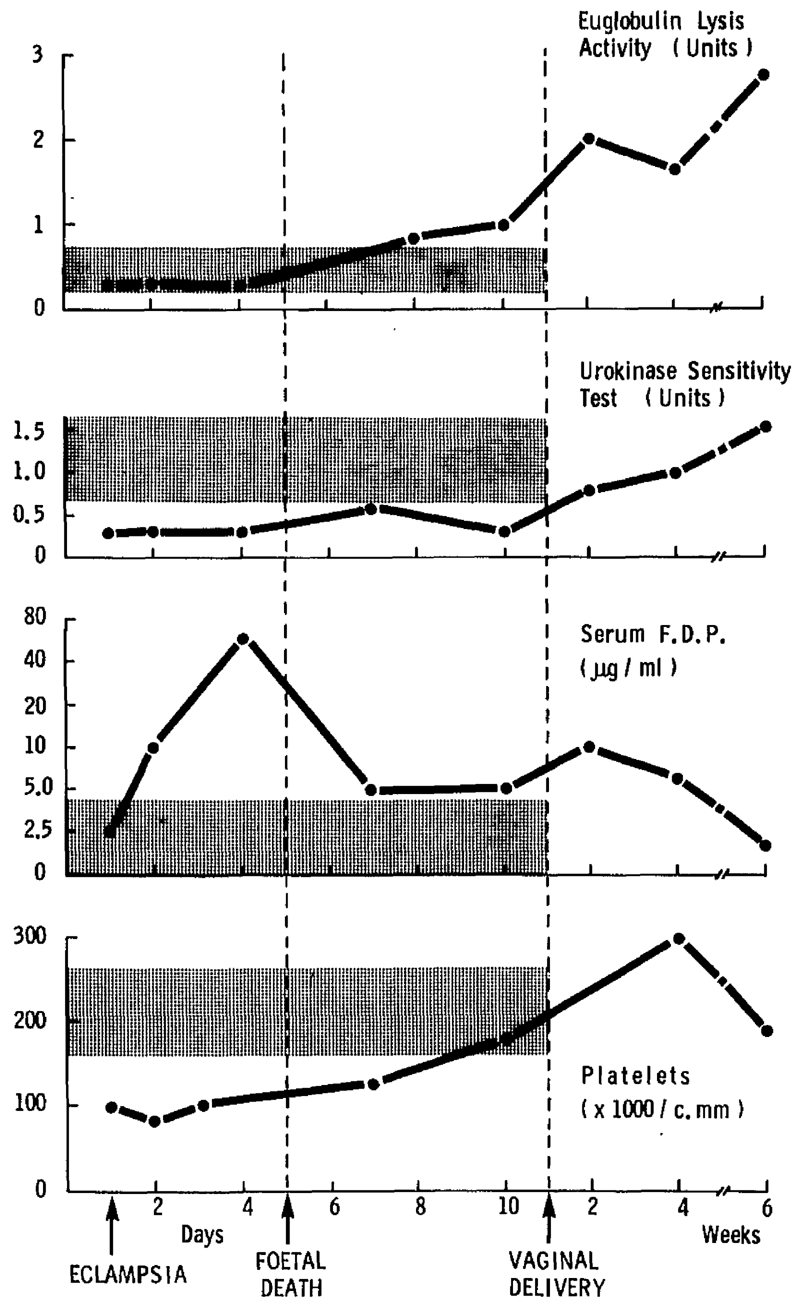

Figure 36

Serial findings in euglobulin lysis activity, urokinase sensitivity test, serum fibrin degradation products (F.D.P.), and platelet count following antepartum eclampsia and foetal death in- utero. The shaded area indicates the range for normal pregnancy prior to delivery.

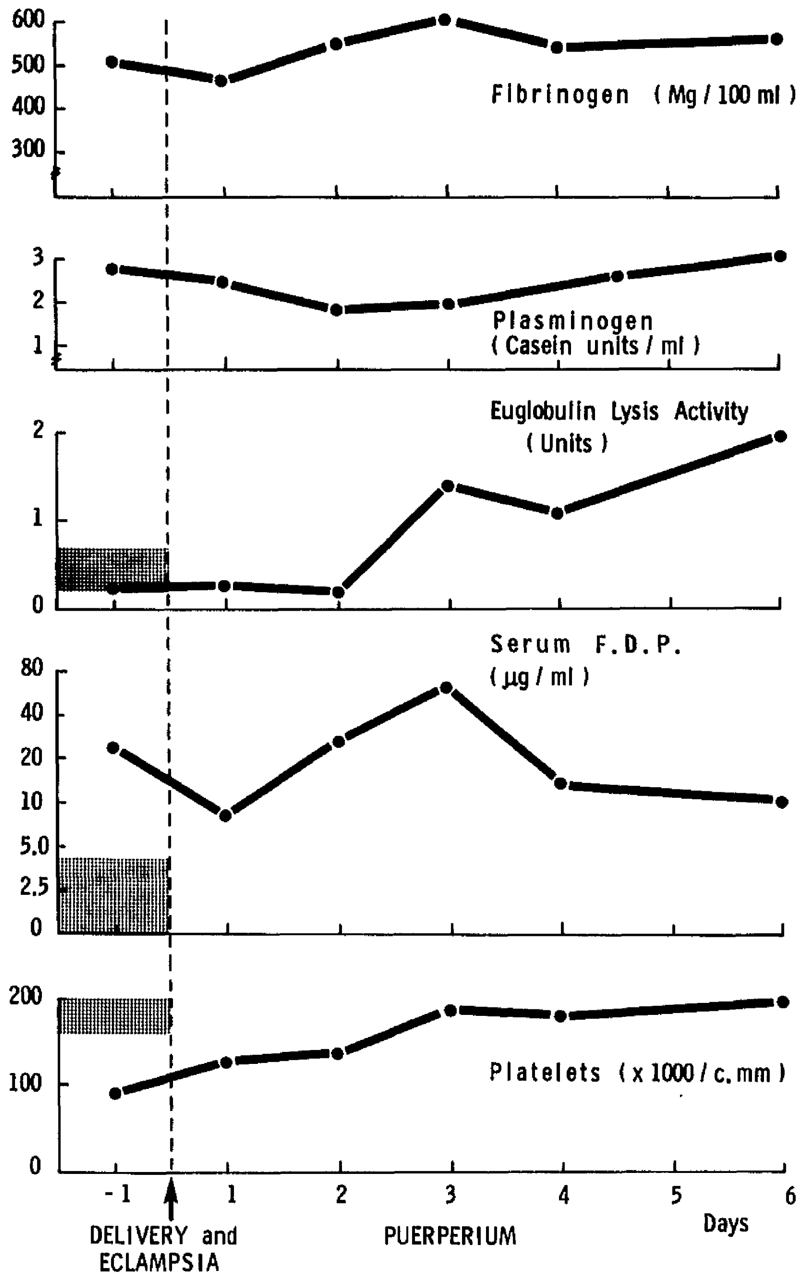

Figure 37

Serial findings before and after postpartum eclampsia in the plasma fibrinogen, plasminogen, euglobulin lysis activity, serum fibrin degradation products (F.D.P.), and platelet count. The shaded area indicates the range for normal pregnancy prior to delivery.

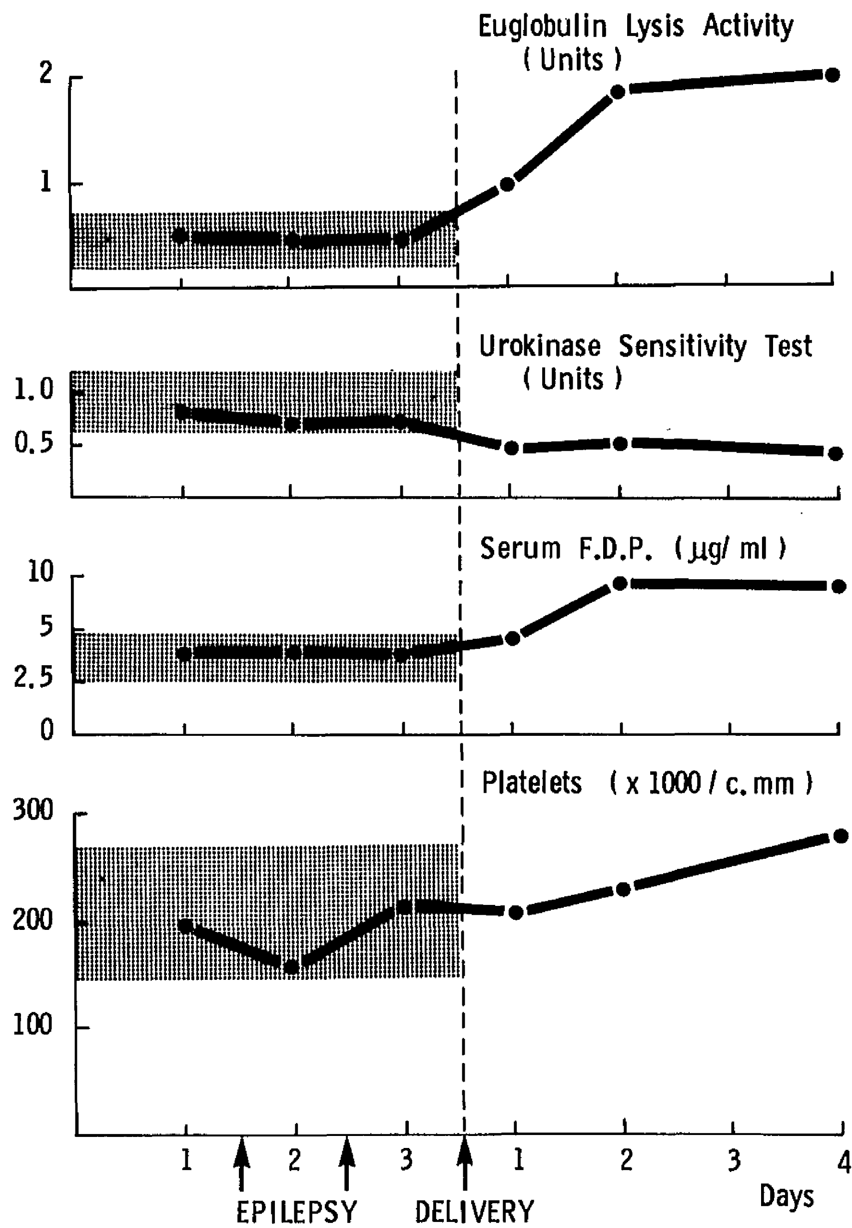

Figure 38

Serial findings in euglobulin lysis activity, urokinase sensitivity test, serum fibrin degradation products (F.D.P.), and platelet count, before and after grand mal seizures at term. The shaded area indicates the range for normal pregnancy prior to delivery.

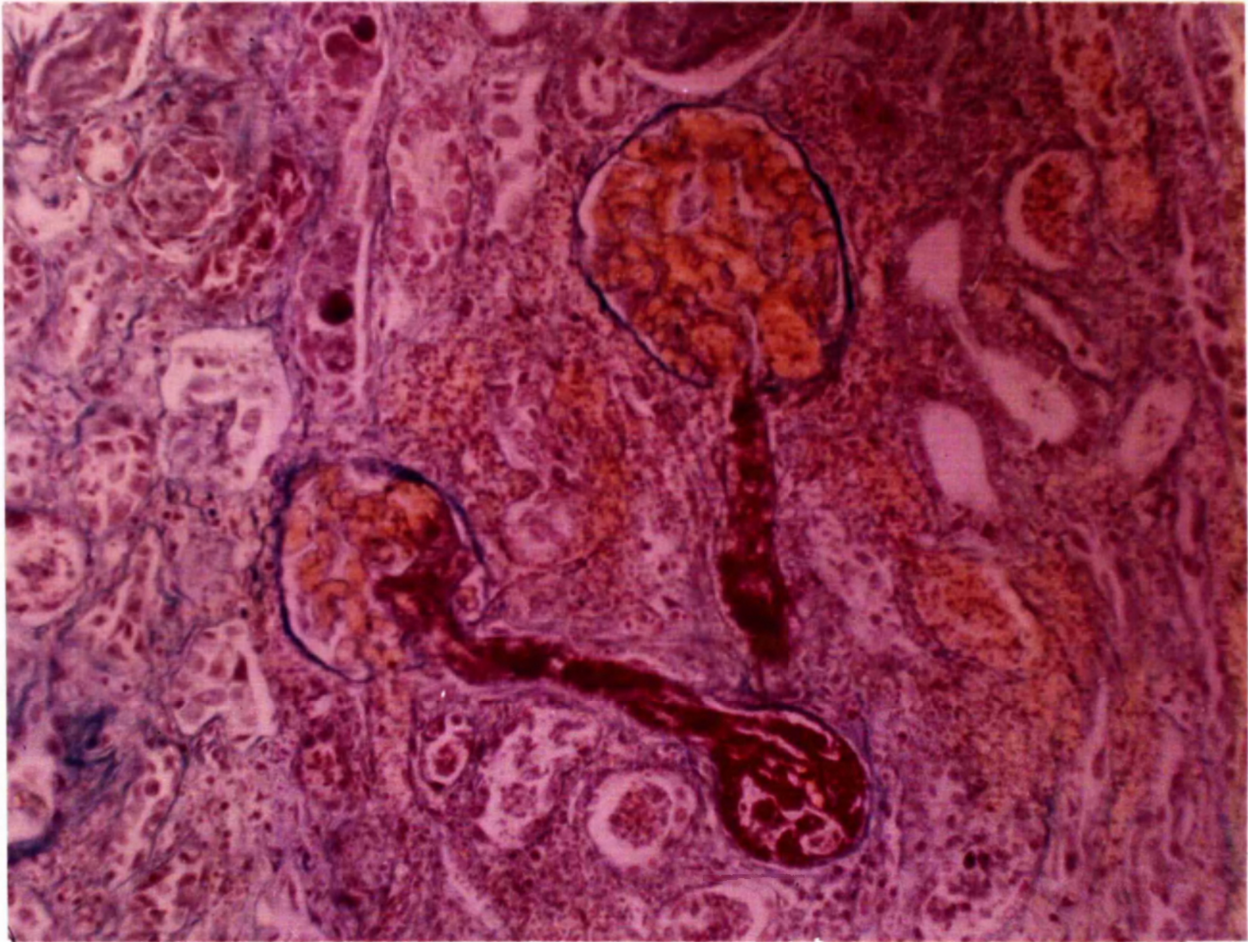

Figure 39

Histological section of the kidney of a patient dying after eclampsia showing fibrin thrombi in the intralobular artery and extending into the afferent arterioles of the glomeruli.
(Picro - Mallory stain ; x 175 diameter).

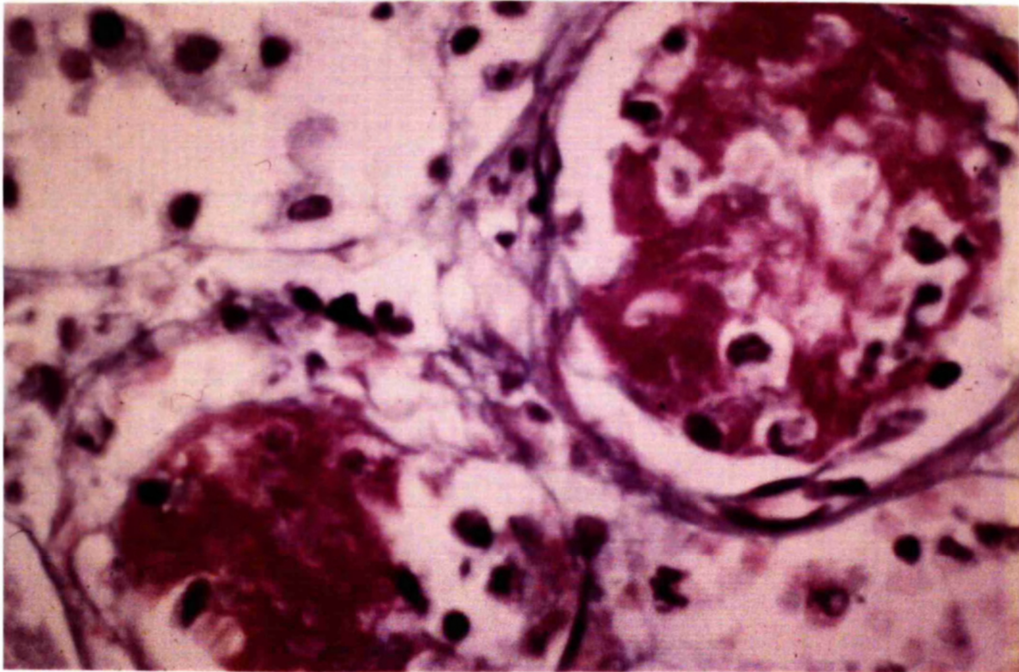

Figure 40

Fibrin fragments within the capillaries of the glomerular tuft in the same patient as figure 39.
(Picro - Mallory stain ; x 325 diameter).

APPENDIX 2.

Tables 1 - 48

Table 1

Fibrinolytic enzyme system in normal pregnancy, labour and the puerperium; Details of patients studied serially

Patient	Age (years)	Parity	Maturity at delivery (weeks)
1	24	0	39
2	21	0	40
3	21	1	40
4	22	2	41
5	27	1	41
6	22	0	40
7	25	2	42
8	30	3	40
9	34	2	38
10	24	0	38
Mean	25	1	40
S.D.	4,5	1	1.3

Table 2

Plasma fibrinogen (mg/ 100ml) during pregnancy, labour and the puerperium

Patient	Gestation (weeks) 12-18	18-24	24-30	30-36	36-42	Labour	Puerperium (1st week)
1	-	320	351	390	390	430	445
2	274	289	303	356	338	382	291
3	360	439	361	333	434	340	427
4	435	440	438	402	476	-	338
5	320	347	358	471	517	508	528
6	324	-	355	550	560	587	478
7	390	-	430	492	492	477	590
8	305	348	-	294	391	379	-
9	406	-	446	449	465	480	450
10	290	303	-	389	373	385	315
Mean	345	355	380	413	443	441	429
S.D.	55.7	61.5	51.4	78.0	70.4	78.6	99.5

Each assay was done in duplicate and the values shown are the average of the two assays. These data are graphically displayed on figure 5.

Table 3

Plasma plasminogen (casein units/ml) during pregnancy, labour and the puerperium

Patient	Gestation (weeks) 12-18	18-24	24-30	30-36	36-42	Labour	Puerperium (1st week)
1	-	4.78	4.98	5.05	4.7	4.7	4.45
2	4.3	4.4	4.3	4.4	3.75	4.05	4.45
3	3.85	-	4.25	5.05	5.37	4.5	5.05
4	5.1	5.18	5.7	4.78	4.82	-	6.35
5	3.25	-	3.4	4.6	4.5	4.75	4.55
6	3.45	-	5.28	5.43	5.65	4.6	5.1
7	-	5.8	5.37	5.33	5.8	5.38	5.4
8	6.1	6.0	6.0	6.05	5.85	6.0	-
9	4.3	-	5.35	5.15	4.4	3.9	3.9
10	4.7	-	5.1	4.8	5.2	3.9	4.1
Mean	4.38	5.23	4.97	5.06	5.00	4.64	4.82
S.D.	0.92	0.67	0.78	0.47	0.69	0.70	0.75

Each assay was done in duplicate and the values shown are the means of the two assays. These data are graphically displayed on figure 5.

Table 4

Euglobulin lysis time (units) during pregnancy, labour and the puerperium

Patient	Gestation (weeks) 12-18	18-24	24-30	30-36	36-42	Labour	Puerperium (1st week)
1	-	0.90	0.30	0.30	0.30	0.30	1.80
2	2.10	0.60	0.30	0.60	0.30	0.60	3.30
3	1.25	1.10	0.90	0.70	0.70	0.80	2.10
4	1.00	0.80	0.30	0.60	0.50	-	1.40
5	1.30	-	0.25	0.25	0.27	-	1.00
6	1.80	-	0.60	0.50	0.30	0.30	1.60
7	-	0.90	0.40	0.20	0.20	0.20	2.20
8	1.43	0.60	-	0.60	0.23	-	-
9	2.10	-	0.50	0.43	0.40	0.30	1.40
10	1.20	-	0.53	0.30	0.30	0.20	1.80
Mean	1.52	0.82	0.45	0.45	0.35	0.39	1.84
S.D.	0.42	0.19	0.21	0.18	0.15	0.27	0.66

Each result is the mean of duplicate assays; patients 4, 5 and 8 delivered shortly after admission and a sample for euglobulin lysis time was not obtained prior to delivery. These data are graphically displayed on figure 7.

Table 5

Urokinase sensitivity test (units) during pregnancy, labour and the puerperium

Patient	Gestation (weeks)					Labour	Puerperium (1st week)
	12-18	18-24	24-30	30-36	36-42		
1	-	2.16	2.34	1.75	1.61	1.64	1.33
2	2.14	2.85	3.15	4.44	2.70	3.50	2.90
3	3.75	3.75	5.40	4.76	5.60	3.50	3.80
4	1.53	1.21	1.50	1.50	1.20	1.18	1.40
5	1.60	2.20	2.30	1.70	1.78	2.60	1.60
6	-	1.80	2.80	2.60	3.06	4.34	3.50
7	-	5.50	4.81	4.80	-	4.50	5.45
8	1.60	-	1.50	1.60	1.50	1.66	-
9	0.92	-	1.90	1.60	1.25	1.58	1.00
10	0.80	-	1.90	2.60	2.85	2.72	2.60
Mean	1.76	2.78	2.76	2.73	2.39	2.72	2.62
S.D.	0.99	1.44	1.35	1.39	1.39	1.20	1.46

Each test was performed in duplicate and the values shown are the average of the two assays. No significant alteration of the urokinase sensitivity test took place during pregnancy, labour and the last week of the puerperium.

Table 6

Thrombin clotting time (seconds) during pregnancy, labour and the puerperium

Patient	Gestation (weeks)					Labour	Puerperium (1st week)
	12-18	18-24	24-30	30-36	36-42		
1	-	12.9	13.3	12.9	11.8	13.1	11.9
2	14.9	14.0	13.6	12.7	13.3	14.0	12.6
3	-	11.0	11.5	11.5	10.9	-	12.4
4	14.6	14.0	13.4	13.4	12.5	12.6	17.0
5	12.4	12.0	11.6	10.8	11.5	13.6	-
6	10.7	-	11.8	12.2	13.8	14.0	12.2
7	11.0	-	10.9	14.5	12.9	16.1	14.3
8	11.9	14.2	14.0	13.2	13.4	13.7	-
9	15.3	14.4	14.0	12.4	14.5	15.0	14.0
10	12.2	12.8	13.4	12.1	13.0	13.6	12.2
Mean	12.9	13.2	12.8	12.6	12.8	13.9	13.3
S.D.	1.81	1.21	1.16	1.02	1.10	1.04	1.73

Each test was done in duplicate and the values shown are the average of the two assays. The times recorded during labour were significantly increased compared with those recorded at 36-42 weeks' gestation ($R = 0$; $p = 0.001$ Wilcoxon's Signed Ranks Test).

Table 7

Platelet counts during pregnancy, labour and the puerperium

Patient	Gestation (weeks) 12-18	18-24	24-30	30-36	36-42	Labour	Puerperium (1st week)
1	245	228	223	225	248	214	262
2	260	226	175	234	239	285	300
3	128	121	136	116	156	101	173
4	223	192	226	271	253	-	456
5	201	184	193	228	238	-	224
6	269	206	250	218	336	326	432
7	160	-	210	198	294	175	252
8	223	172	244	195	180	115	-
9	235	-	210	184	192	148	385
10	213	-	247	204	162	150	198
Mean	216	190	211	206	230	189	298
S.D.	43.7	36.8	35.8	42.8	58.1	80.4	103.0

Platelet counts (x1000) per cmm are shown; each figure is the mean of duplicate counts; the increase of the platelet count during the first week of the puerperium is highly significant ($R = 0$; $p = 0.001$ Wilcoxon's Signed Ranks Test).
These data are graphically displayed on figure 8.

Table 8

Fibrinolytic enzyme system in pregnancy, control patients: Fibrinogen, plasminogen, euglobulin lysis time, urokinase sensitivity test, thrombin clotting time and platelet count

Patient	Fibrinogen (mg/100ml)	Plasminogen (units/ml)	Euglobulin lysis time (units)	Urokinase sensitivity test (units)	Thrombin time (seconds)	Platelet count ($X10^3$/cmm)
1	328	2.50	1.42	0.82	10.5	296
2	474	2.35	1.50	0.60	9.5	215
3	381	3.00	0.28	2.13	10.0	142
4	351	2.40	1.58	0.57	11.5	359
5	327	3.55	0.77	0.76	12.0	272
6	355	3.25	2.00	0.41	11.8	214
7	363	2.55	1.00	1.48	12.0	192
8	339	2.65	3.33	1.55	11.5	142
9	418	2.75	0.91	1.54	13.0	298
10	254	3.15	1.18	1.07	11.8	198
11	293	4.05	1.00	1.93	12.5	278
12	292	3.50	1.00	1.53	12.8	444
13	187	2.65	2.50	1.53	13.5	313
14	280	3.35	1.53	1.76	13.5	188
15	195	3.80	0.91	0.64	15.0	296
16	130	2.90	2.85	1.69	14.0	231
17	198	3.50	0.55	1.43	12.8	264
18	255	4.10	1.25	1.81	15.0	215
19	148	2.90	0.74	1.64	13.5	202
20	191	3.15	1.00	1.63	12.5	207

Table 8 (continued)

Patient	Fibrinogen (mg/100ml)	Plasminogen (units/ml)	Euglobulin lysis time (units)	Urokinase sensitivity test (units)	Thrombin time (seconds)	Platelet count ($X10^3$/cmm)
21	133	1.60	1.82	1.48	12.5	295
22	297	2.90	0.87	1.41	10.5	234
23	308	2.50	0.80	1.50	11.5	290
24	181	2.00	1.67	2.17	13.2	239
25	135	2.40	0.64	1.55	10.5	212
26	135	2.00	2.14	2.30	14.5	402
27	286	2.65	2.00	1.82	11.0	187
28	229	1.75	1.00	2.42	13.0	272
29	272	2.25	1.67	2.02	10.5	214
30	310	3.00	1.82	1.65	14.8	192
Mean	268	2.84	1.39	1.50	12.4	250
S.D.	90.7	0.63	0.70	0.52	1.5	69.6

The results of the assays in 30 healthy women examined approximately 8 weeks after a normal pregnancy and spontaneous delivery.

Table 9

Serum fibrin/fibrinogen degradation products (FDP) and pregnancy

	Gestation (weeks)					Labour	Puerperium	
	<18	18-24	24-30	30-36	36-Term		1st week	6th week
Mean level of FDP + S.D. (μg/ml)	1.05 ± 0.36	1.25 ± 0.53	1.41 ± 0.78	1.31 ± 1.0	1.26 ± 0.54	3.45 ± 2.8	4.2 ± 3.2	1.86 ± 1.7
No. of patients	14	26	25	25	26	75	24	35

The levels of fibrin/fibrinogen degradation products (FDP) found in the serum of 250 patients investigated during pregnancy, labour and the puerperium. The increase of the levels during labour compared with the levels found in the 3rd trimester is highly significant ($t = 4.555$; $p<0.001$). The individual values and the data in this table are graphically displayed in figure 9.

Table 10

Serum fibrin/fibrinogen degradation products (FDP) following caesarean section

Patient	Post Operation 2-4 hours	3-8 days
1	7.2	14.4
2	7.2	3.6
3	2.2	35.2
4	2.5	20.0
5	1.6	108.0
6	20.0	20.0
7	1.6	6.0
8	3.0	1.5
9	3.6	5.0
10	10.0	26.0
11	3.6	6.4
12	7.6	6.4
Mean	5.8	21.0
S.D.	5.2	29.3

Shown are the levels of fibrin/fibrinogen degradation products (FDP) in μg/ml of serum in 12 patients who had blood samples taken 2-4 hours following delivery by caesarean section and again 3-8 days after operation. The increase in the levels of FDP is significant ($R = 9$, $p<0.05$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 10.

Table 11

Plasma recalcification time (minutes)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	6.9	6.7	5.4	5.2	6.4	8.1	6.2
2	7.4	-	5.5	6.3	5.3	8.8	5.0
3	5.9	5.2	4.7	5.3	4.3	6.3	-
4	5.5	4.0	3.9	4.1	4.8	5.9	-
5	6.2	6.2	5.0	4.9	7.3	5.7	-
6	7.2	5.5	5.4	5.8	6.6	7.7	-
7	8.2	5.8	-	7.2	6.1	6.9	-
8	6.7	6.1	5.5	5.8	6.7	5.5	5.8
9	9.5	4.6	4.5	2.6	4.5	5.1	5.2
10	6.9	5.1	6.1	-	6.5	5.2	5.4
11	4.2	3.8	3.5	-	-	5.8	4.6
12	5.2	-	-	3.7	5.2	6.4	-
13	7.9	7.9	7.6	7.7	5.7	5.8	-
14	7.0	5.4	6.3	5.3	6.6	6.0	5.8
15	6.9	5.9	5.3	6.2	5.7	8.0	6.1
Mean	6.8	5.5	5.3	5.4	5.8	6.5	5.5
S.D.	1.3	1.1	1.1	1.4	0.9	1.2	0.6

Shown are the plasma recalcification times (minutes) performed in plastic tubes in serial samples of peripheral blood during and after spontaneous vaginal delivery. The blood sample before delivery (2nd stage of labour) was taken when the baby's head was distending the perineum; the sample for the 3rd stage was taken immediately following delivery of the infant while the placenta was separating 'in utero'; the samples after placental delivery were taken within 1 minute of expulsion of the placenta and after an interval of 15 minutes and 1 hour. The shortening of the plasma recalcification time during the 3rd stage of labour and within one minute of placental delivery was highly significant ($R = 0$, $p<0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 11.

Table 12

Kaolin-cephalin clotting time (seconds)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	45	47	44	44	46	45	45
2	35	-	35	35	40	32	35
3	38	39	37	40	39	40	-
4	45	45	43	43	44	44	-
5	52	41	50	47	54	41	-
6	38	38	39	41	44	45	-
7	42	30	-	38	37	-	-
8	46	42	44	43	43	42	41
9	43	43	43	34	41	44	41
10	46	48	48	-	46	41	-
11	49	38	37	-	41	40	47
12	43	45	42	46	43	46	32
13	42	42	43	41	40	42	-
14	50	49	45	49	46	50	45
15	40	40	39	39	43	42	37
Mean	44	42	42	42	43	42	40
S.D.	5	5	4	4	4	4	5

Shown are the kaolin-cephalin clotting times (seconds) in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11. When the kaolin cephalin clotting time immediately prior to delivery (2nd stage) is compared with the lower of the values recorded during or immediately after placental separation (3rd stage and within 1 minute of placental delivery) the decrease is significant ($R = 6.5$, $p<0.05$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 11.

Table 13

Partial thromboplastin time (seconds)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	51	39	37	-	53	55	56
2	73	-	63	80	76	54	67
3	71	69	69	69	70	74	-
4	65	60	56	61	67	57	-
5	69	60	62	68	69	67	-
6	57	47	45	52	66	61	-
7	74	57	-	66	67	66	-
8	59	57	54	56	56	58	63
9	60	56	54	47	46	54	62
10	48	58	52	-	53	52	56
11	56	62	51	51	59	52	51
12	50	51	49	60	49	50	50
13	48	53	45	55	49	58	-
14	51	48	56	51	49	60	58
15	62	58	59	61	66	54	58
Mean	60	55	54	60	60	58	58
S.D.	9	7	8	9	10	7	6

Shown are the partial thromboplastin times (seconds) in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11. When the partial thromboplastin time immediately prior to delivery (2nd stage) is compared to the value recorded immediately after delivery of the placenta (1 minute) the decrease is highly significant ($R = 11$, $p<0.01$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 11.

Table 14

Factor II (prothrombin) levels
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	100	100	105	125	135	115	120
2	200	-	215	185	200	155	130
3	165	135	145	155	155	130	-
4	105	115	112	115	112	105	-
5	130	150	130	130	120	98	-
6	195	190	210	170	160	130	-
7	100	150	-	150	140	-	-
8	100	135	130	125	160	145	200
9	120	125	150	150	120	105	130
10	140	140	160	-	200	114	80
11	94	100	104	-	100	124	100
12	192	-	-	150	250	130	-
13	180	180	190	190	210	180	-
14	130	130	115	145	140	76	135
15	160	160	175	150	175	145	120
Mean	141	139	149	149	158	125	127
S.D.	38	27	38	23	42	26	35

Shown are the percentage levels of factor II (prothrombin) in the peripheral blood during and after spontaneous vaginal delivery. The timing of the blood samples was as recorded in the legend to Table 11. The level of factor II on the first day of the puerperium was significantly lower than the levels recorded during placental delivery ($p<0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 12.

Table 15

Factor V levels during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	58	66	70	70	84	76	140
2	180	-	360	310	300	120	105
3	90	70	79	70	72	96	-
4	86	100	100	170	120	90	88
5	110	85	85	110	85	-	110
6	100	100	140	100	100	180	-
7	160	160	-	130	130	100	-
8	86	150	130	110	130	130	140
9	88	88	90	98	115	105	160
10	100	100	100	-	156	72	70
11	74	132	140	114	144	-	128
12	250	-	-	300	300	120	-
13	215	205	205	210	205	120	-
14	180	180	190	180	180	130	130
15	230	210	230	180	180	140	230
Mean	134	127	148	154	153	114	130
S.D.	63	50	82	77	73	29	44

Shown are the percentage levels of factor V in the peripheral blood during and after spontaneous vaginal delivery. The timing of the blood specimens was as recorded in the legend to Table 11. The decrease of factor V between 1 hour of placental delivery and the first day of the puerperium is significant ($R = 11$, $p<0.05$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 12.

Table 16

Factor VIII levels during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	170	170	205	185	145	110	215
2	216	-	264	312	240	312	320
3	192	198	198	198	115	240	-
4	335	308	364	378	343	-	294
5	154	137	154	143	154	196	-
6	186	226	284	186	119	-	132
7	216	528	-	240	216	276	-
8	235	222	280	210	205	130	145
9	250	250	220	295	280	110	138
10	230	250	190	-	160	170	100
11	220	320	470	-	185	320	310
12	190	-	-	140	230	210	-
13	230	220	220	220	215	180	-
14	165	175	165	158	168	131	137
15	189	203	245	238	308	280	196
Mean	212	247	251	223	206	205	199
S.D.	44	99	87	70	67	75	82

Shown are the percentage levels of factor VIII (antihaemophilic factor) in the peripheral blood during and after spontaneous vaginal delivery. The timing of the blood samples was as recorded in the legend to Table 11. When the level of factor VIII immediately prior to delivery of the baby (2nd stage) is compared to the peak level of factor VIII recorded during or immediately after placental delivery (3rd stage and 1 minute) the increase of factor VIII is highly significant ($R = 2.5$, $p<0.01$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 12.

Table 17

Factor IX levels during and after normal delivery

Patient	Labour (stage) 2nd	Labour (stage) 3rd	Placental Delivery (minutes) 1	Placental Delivery (minutes) +15	Placental Delivery (minutes) +60	Puerperium (days) 1st	Puerperium (days) 3-5
1	79	84	81	83	68	130	160
2	85	-	100	145	100	170	165
3	82	94	94	120	125	170	-
4	130	140	130	125	105	90	190
5	66	64	74	55	80	76	100
6	133	160	130	115	135	210	98
7	80	95	95	-	140	100	90
8	-	-	-	-	-	120	90
9	120	-	-	140	110	170	-
10	180	160	138	160	93	108	-
11	82	74	83	64	66	88	100
12	76	76	68	47	100	140	125
Mean	101	105	99	105	102	131	124
S.D.	35	38	25	40	25	42	38

Shown are the percentage levels of factor IX in peripheral blood during and after spontaneous vaginal delivery. The timing of the blood samples was as recorded in the legend to Table 11. The serial assays were performed in 12 of the 15 patients in this group.

The data are graphically displayed in figure 12.

Table 18

Factor X levels during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	140	170	110	100	170	95	120
2	185	-	185	125	100	190	130
3	135	115	100	135	100	80	-
4	130	115	130	130	130	175	-
5	240	250	240	250	200	190	-
6	180	110	150	150	170	90	-
7	210	170	-	190	210	-	-
8	140	180	140	-	140	200	120
9	140	180	180	-	140	180	120
10	180	180	190	170	170	125	-
11	120	120	120	94	130	180	100
12	189	203	245	238	308	280	94
Mean	166	163	163	158	164	162	112
S.D.	37	44	50	54	57	60	14

Shown are the percentage levels of factor X in the peripheral blood during and after spontaneous vaginal delivery. The timing of the blood samples was as recorded in the legend to Table 11. The serial assays were performed in 12 of the 15 patients in this group.

The data are graphically displayed in figure 12.

Table 19

One stage prothrombin time (seconds)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	12.5	12.5	12.0	12.5	12.5	13.0	13.0
2	13.0	-	12.5	11.5	12.0	13.5	12.0
3	13.0	13.0	13.0	13.0	12.5	12.0	-
4	12.0	11.0	11.0	11.0	13.0	11.5	-
5	11.0	10.5	11.5	11.0	12.5	12.5	-
6	12.5	13.0	12.0	13.5	14.0	12.5	-
7	15.5	13.0	-	12.5	13.5	15.0	-
8	15.0	14.5	13.5	15.0	14.0	12.0	14.0
9	15.5	15.0	14.5	14.5	14.5	15.0	14.0
10	15.0	15.0	16.0	-	15.0	15.0	13.0
11	13.5	13.0	13.0	-	14.5	14.5	13.0
12	13.5	-	-	14.0	13.5	13.0	-
13	12.0	12.0	12.0	12.0	12.0	12.5	-
14	14.0	14.0	15.0	14.5	14.0	15.0	15.0
15	11.5	12.0	12.5	12.0	12.0	13.0	12.0
Mean	13.3	12.9	12.9	12.8	13.3	13.3	13.3
S.D.	1.4	1.4	1.5	1.4	1.0	1.2	1.0

Shown are the one stage prothrombin times (seconds) in the peripheral blood during and after spontaneous vaginal delivery. The timing of the samples was as recorded in the legend to Table 11. The slight decrease of the prothrombin time in the 3rd stage of labour and following placental delivery was not significant.

The data are graphically displayed in figure 13.

Table 20

Thrombin clotting time (seconds)
during and after normal delivery

Patient	Labour (stage) 2nd	Labour (stage) 3rd	Placental Delivery (minutes) 1	Placental Delivery (minutes) +15	Placental Delivery (minutes) +60	Puerperium (days) 1st	Puerperium (days) 3-5
1	10.0	9.5	10.0	9.5	10.0	9.0	10.0
2	8.0	-	8.0	9.5	9.0	10.0	10.0
3	11.0	11.5	10.5	11.0	9.5	9.5	-
4	10.5	10.0	10.0	10.0	10.0	10.0	9.0
5	10.5	10.0	10.0	10.0	10.0	11.0	-
6	9.5	10.0	10.0	11.0	10.0	9.0	-
7	10.0	10.5	-	10.0	11.0	-	-
8	11.0	10.5	9.0	11.0	10.5	8.5	8.5
9	10.0	10.5	10.5	10.0	10.0	11.0	11.0
10	11.5	-	-	11.0	10.5	10.0	-
11	10.5	10.5	11.5	11.5	10.0	10.5	-
12	8.5	9.0	9.0	9.0	10.0	9.5	-
13	10.5	10.0	10.0	10.5	10.0	10.0	10.5
Mean	10.1	10.2	9.9	10.3	10.0	9.8	9.8
S.D.	1.0	0.7	0.9	0.8	0.5	0.8	0.9

Shown are the thrombin clotting times in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11. The serial thrombin clotting times were performed in 13 of the 15 patients in this group and no significant change was found during or immediately following delivery.

The data are graphically displayed in figure 13.

Table 21

Platelet counts (x 1000) during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	-	202	185	217	176	214	285
2	261	-	278	288	239	255	283
3	-	260	279	316	290	281	-
4	252	258	267	275	269	294	-
5	141	192	178	184	250	240	295
6	235	184	172	173	-	190	-
7	211	100	-	145	168	201	-
8	269	312	241	197	190	233	291
9	168	238	195	158	174	214	329
10	195	179	180	-	163	244	273
11	221	182	180	-	199	273	337
12	240	217	241	254	255	239	333
13	184	205	166	202	202	160	-
14	-	308	251	-	263	254	285
15	271	261	240	237	206	274	332
Mean	221	221	218	220	217	238	304
S.D.	42	56	42	54	42	37	25

Shown are the platelet counts (x 1000 per cmm) in the peripheral blood during and after spontaneous vaginal delivery. The timing of the blood samples was as recorded in the legend to Table . The increase of the platelet count 3-5 days after delivery was highly significant ($R = 0$, $p<0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 13.

Table 22

Plasma fibrinogen levels (mg/ 100ml)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	419	372	479	399	439	405	379
2	480	-	330	414	435	382	-
3	351	363	432	321	316	363	-
4	320	334	251	261	277	293	-
5	353	311	330	314	318	-	-
6	434	434	-	347	335	422	-
7	382	342	382	324	339	399	528
8	470	477	378	354	443	413	481
9	428	411	428	-	375	390	-
10	337	368	351	-	419	390	368
11	472	-	-	426	431	448	-
12	281	270	273	275	343	326	-
13	374	374	298	286	333	204	380
14	386	315	315	287	331	408	376
15	350	365	324	318	376	290	456
Mean	389	364	352	333	367	374	424
S.D.	60	54	66	53	54	51	64

Shown are the levels of plasma fibrinogen (mg/ 100ml) in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11. The decrease of the plasma fibrinogen level between the 2nd stage of labour and 15 minutes after placental delivery is highly significant ($R = 0$, $p<0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 14.

Table 23

Plasma plasminogen levels (casein units/ml)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	2.8	3.0	2.7	2.9	2.9	2.6	3.0
2	3.2	-	3.0	3.0	3.0	2.9	-
3	2.3	2.2	2.0	2.5	2.3	2.4	-
4	2.5	2.7	3.0	2.3	2.7	2.2	-
5	2.5	1.8	-	2.2	2.6	2.9	-
6	4.1	3.1	-	3.2	3.0	2.8	-
7	2.7	2.5	3.0	2.6	2.7	2.3	2.3
8	2.2	3.1	2.3	-	2.3	2.1	2.8
9	3.3	3.2	3.0	-	3.2	2.8	-
10	2.4	2.2	2.2	-	2.3	2.3	2.3
11	4.9	-	-	5.7	5.0	5.2	-
12	2.6	2.3	2.6	2.4	2.6	2.3	-
13	3.5	3.4	3.1	3.1	3.3	3.3	2.3
14	3.3	3.3	3.4	3.5	3.5	2.4	2.7
15	3.2	3.3	3.2	3.1	3.1	2.8	2.4
Mean	3.03	2.78	2.78	3.04	2.97	2.75	2.54
S.D.	0.74	0.52	0.43	0.93	0.67	0.75	0.29

Shown are the plasma plasminogen levels (casein units/ml) in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11. If the lower level of plasminogen recorded between the 3rd stage and 1 minute of placental separation is compared with the plasminogen level in the 2nd stage the decrease is significant ($R = 8.5$, $p<0.01$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 14.

Table 24

Plasminogen activator levels (euglobulin lysis time) during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	0.29	0.30	0.29	0.33	0.91	1.25	1.11
2	0.35	-	0.35	0.35	0.85	0.73	1.15
3	0.40	0.43	0.43	0.37	0.50	1.00	-
4	0.43	0.40	0.43	0.45	1.67	1.43	-
5	0.33	0.33	0.50	0.71	1.82	1.50	-
6	0.45	0.36	-	0.71	1.67	-	-
7	0.28	0.33	0.73	0.50	1.20	2.50	1.11
8	0.15	0.20	0.16	0.15	1.43	0.56	1.43
9	0.23	0.33	0.30	-	1.87	1.15	1.43
10	0.37	0.32	0.42	-	2.00	1.43	2.20
11	0.25	-	-	0.45	1.43	1.36	-
12	0.31	0.36	0.50	0.88	1.02	1.00	-
13	0.38	0.50	0.63	0.59	1.11	1.67	1.67
14	0.50	0.62	0.56	0.62	1.24	1.00	2.00
Mean	0.34	0.37	0.44	0.59	1.34	1.28	1.51
S.D.	0.09	0.11	0.16	0.44	0.44	0.48	0.42

Shown are the levels of plasminogen activator expressed in units, calculated from the reciprocal of the euglobulin lysis time as described in the text (page 29). The timing of the blood samples was as recorded in the legend to Table 11. The increase in the level of plasminogen activator 1 hour after delivery of the placenta is highly significant ($R = 0$, $p<0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 14.

Table 25

Urokinase sensitivity test (units)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	0.51	0.66	0.54	0.51	0.46	0.77	1.02
2	0.24	-	0.60	0.43	0.57	0.29	0.56
3	1.03	1.37	0.98	1.08	0.69	0.59	-
4	0.66	0.50	0.49	0.55	0.50	0.17	-
5	0.73	0.87	0.72	0.73	0.69	0.51	-
6	1.17	0.87	1.04	0.69	0.80	-	0.77
7	0.74	0.77	-	0.59	0.59	0.52	0.95
8	1.15	0.90	1.46	1.04	1.01	0.72	0.71
9	1.27	1.23	1.17	0.96	1.18	0.84	1.30
10	1.66	1.25	1.19	-	1.07	0.73	0.56
11	0.86	1.05	1.29	-	1.15	0.60	0.95
12	1.70	-	-	2.23	1.76	1.35	-
13	0.97	0.95	0.86	0.68	0.88	0.29	-
14	1.38	1.18	0.91	1.02	0.93	1.15	0.83
15	0.87	0.76	0.61	0.66	0.69	0.55	0.54
Mean	1.00	0.95	0.91	0.86	0.86	0.66	0.82
S.D.	0.41	0.25	0.31	0.46	0.34	0.31	0.24

Shown are the serial results of the urokinase sensitivity test expressed in units calculated from the reciprocal of the clot lysis time as described in the text on page . The timing of the blood samples was as recorded in the legend to Table 11. On the first day of the puerperium the sensitivity to urokinase induced lysis was significantly less than that recorded prior to delivery (2nd stage), i.e. the inhibition to urokinase induced lysis was greater on the first day after delivery than before delivery ($R = 6.5$, $p = 0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 14.

Table 26

Fibrin degradation products (µg/ml serum)
during and after normal delivery

Patient	Labour (stage)		Placental Delivery (minutes)			Puerperium (days)	
	2nd	3rd	1	+15	+60	1st	3-5
1	11.2	5.6	5.6	5.6	5.6	1.4	1.4
2	1.1	-	1.1	4.4	8.8	8.8	2.2
3	4.0	4.0	4.0	8.0	8.0	4.0	-
4	2.8	1.4	1.4	1.4	1.4	1.4	-
5	1.2	1.2	1.2	1.2	2.4	2.4	-
6	1.4	1.4	-	1.4	1.4	5.6	-
7	7.2	7.2	7.2	-	14.4	3.6	3.6
8	2.6	2.6	2.6	-	2.6	2.6	1.8
9	1.7	2.6	2.6	-	2.6	2.6	2.6
10	3.4	-	-	3.4	13.5	10.1	-
11	5.6	5.6	1.4	11.2	11.2	5.6	-
12	2.0	2.0	2.0	2.0	2.0	1.0	1.0
13	2.2	1.1	1.1	1.1	1.1	8.8	4.4
14	6.8	6.8	6.8	6.8	6.8	6.8	3.4
Mean	3.80	3.46	3.08	4.23	5.84	4.62	2.55
S.D.	2.93	2.28	2.27	3.35	4.68	3.04	1.18

Shown are the levels of serum fibrin/fibrinogen degradation products (µg/ml) in serial samples of peripheral blood during and after spontaneous vaginal delivery. The timing of the specimens was as recorded in the legend to Table 11.

The data are graphically displayed in figure 14.

Table 27

Whole blood clotting time in plastic (minutes) in uterine vein blood and peripheral vein blood during and after placental separation at caesarean section

Patient	Uterine Blood				Peripheral Blood				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation
1	-	2	-	8	14	13	20	22	19
2	23	1.5	7.5	18	21	19	20	28	24
3	-	2.5	17	7	22	17	18	-	17
4	-	0.5	-	-	-	-	-	-	-
5	39	28	14	-	31	33	26	-	15
6	16	1.5	1	26	25	20	-	-	20
7	17	1.5	3	20	18	22	-	-	32
8	-	12	-	15	-	17	15	-	-
9	14	-	28	28	26	-	23	28	-
10	26	11	15	26	26	23	23	22	-
11	17	-	12	15	16	18	-	15	23
12	15	21	15	-	22	-	22	-	-
Mean	21	8	12	18	22	20	21	23	21
S.D.	8	9	8	8	5	6	3	5	6

The timing of the blood samples was as recorded in legend to Table 37.

The whole blood clotting time in plastic in the sample from the uterine vein during placental separation was highly significantly shorter than the time recorded in the simultaneous sample from the arm vein ($R = 0$; $p<0.01$; Wilcoxon's Signed Ranks Test).

The data are graphically displayed in figure 16.

Table 28

Plasma recalcification time in plastic (minutes) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

	Uterine Blood				Peripheral Blood					Post Operation				
atient	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	3.1	4.8	5.1	6.8	5.9	6.8	6.2	6.2	5.1	6.3	6.2	-	6.7
2	6.0	4.7	5.1	6.1	7.5	5.2	5.4	5.2	5.8	7.1	-	-	7.2	5.0
3	-	2.8	5.3	4.5	6.0	7.8	6.1	-	5.6	5.5	5.8	8.1	10.2	7.1
4	-	-	-	4.5	-	5.2	4.5	5.1	4.1	-	7.5	4.0	6.1	3.5
5	-	3.7	3.7	-	3.8	-	-	3.8	-	-	6.8	7.0	-	-
6	7.4	5.8	4.4	-	6.0	6.5	5.7	5.2	-	5.9	7.1	-	7.0	-
7	5.0	-	3.4	-	5.9	4.2	-	-	6.1	6.7	4.1	-	4.2	-
8	3.2	3.2	3.1	-	3.1	3.2	-	-	3.5	-	-	4.1	-	-
9	6.1	4.0	5.0	6.8	7.0	4.5	6.8	6.5	-	5.7	4.8	4.7	7.0	6.8
10	5.8	-	4.2	4.7	7.0	5.2	5.7	6.4	-	5.1	6.3	6.6	7.0	-
11	8.8	6.8	5.4	5.5	8.5	7.0	6.8	4.8	8.0	5.2	7.2	8.1	5.5	6.2
12	6.5	6.9	5.5	6.0	8.3	8.5	5.6	6.8	-	4.8	7.7	5.8	7.4	-
ean	6.1	4.5	4.5	5.4	6.3	5.7	5.9	5.6	5.6	5.7	6.3	6.1	6.8	5.9
.D.	1.6	1.5	0.8	0.8	1.8	1.6	0.7	1.0	1.5	0.8	1.2	1.6	1.6	1.4

The timing of the blood samples was as recorded in the legend to Table 37.

The shortening of the plasma recalcification time in uterine vein blood during placental separation was highly significant ($t = 3.5$, $p<0.01$) and the recalcification time in the uterine blood was significantly less than that in the peripheral blood sample taken simultaneously ($R = 0$, $p = 0.01$).

The data are graphically displayed in figure 16.

Table 29

Platelet counts in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

	Uterine Blood				Peripheral Blood					Post Operation				
atient	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	331	318	310	315	375	313	374	342	293	310	312	528	575
2	244	-	263	201	207	237	210	210	209	256	-	201	307	348
3	-	116	137	-	136	121	151	128	-	147	197	227	231	-
4	-	-	-	247	200	265	259	244	239	-	280	362	530	698
5	357	302	322	-	310	-	385	-	-	297	336	484	484	705
6	315	-	-	325	351	-	303	-	-	361	395	324	-	389
7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9	165	149	145	160	176	-	155	151	-	197	206	274	242	387
10	172	130	176	-	160	181	155	164	-	175	246	300	311	-
11	140	145	130	158	150	170	163	166	142	115	-	298	266	332
12	200	189	236	236	225	209	197	198	-	216	173	358	-	394
ean	228	195	216	234	223	222	229	204	233	229	268	310	362	478
.D.	82	87	80	67	76	82	82	78	83	77	76	76	129	156

The timing of the blood samples was as recorded in the legend to Table 37.

The platelet counts in patients 7 and 8 have been excluded as only a small number were available. At 10-14 days after caesarean section the platelet counts were highly significantly increased from the levels at 3-5 days after operation ($R = 0$; $p = 0.002$) and the platelet counts in the control patients (Table 8) ($t = 6.2$, $p<0.001$).

The data are graphically displayed in figure 16.

Table 30

Kaolin cephalin clotting time (seconds) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	41	42	44	49	42	47	46	44	42	-	-	44	41
2	46	40	40	44	45	43	46	46	47	50	-	47	49	54
3	-	31	36	35	38	42	39	-	37	35	39	39	37	42
4	-	-	-	53	-	54	56	60	61	-	71	65	45	39
5	41	38	38	-	40	41	38	-	-	37	44	40	-	-
6	53	-	-	46	49	-	46	-	-	46	45	-	-	-
7	46	41	41	-	44	41	-	-	42	-	-	42	-	-
8	48	43	43	44	46	42	42	46	47	46	43	-	43	42
9	55	-	56	59	61	-	60	58	-	51	48	41	53	39
10	43	42	42	40	45	44	41	44	-	48	42	41	40	40
11	35	40	41	41	44	39	47	44	41	41	46	42	43	38
12	36	35	37	35	38	37	37	36	-	36	38	34	39	-
ean	44.8	39.0	41.6	44.1	45.4	42.5	45.4	47.5	45.6	43.2	46.2	43.4	43.7	43.1
D.	6.9	3.8	5.6	7.4	6.4	4.5	7.3	7.8	7.7	5.9	9.8	8.8	5.0	6.2

The timing of the blood samples was as shown in the legend to Table 37.

The data are graphically displayed in figure 17.

Table 31

Partial thromboplastin time (seconds) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	50	61	56	58	53	52	57	55	65	70	57	57	60
2	53	55	54	53	56	54	55	54	52	63	-	72	68	71
3	-	53	56	58	63	64	59	-	56	53	58	58	60	-
4	58	53	55	58	59	58	59	56	56	-	56	56	58	60
5	54	41	42	-	51	53	48	-	-	57	53	56	68	-
6	56	-	-	53	55	-	50	-	56	52	57	-	-	-
7	81	72	71	-	83	62	-	-	66	-	-	53	-	-
8	76	73	69	68	-	67	65	61	64	62	69	68	67	66
9	66	-	72	77	82	-	80	71	-	64	53	62	57	54
10	65	55	60	59	65	65	65	62	-	69	61	54	61	-
11	77	72	62	61	80	66	63	61	67	73	71	73	73	87
12	59	54	53	58	65	57	61	55	-	58	62	53	58	-
Mean	65	58	60	60	65	60	60	60	59	62	61	60	63	66
.D.	10	11	9	7	11	6	9	6	6	7	7	7	6	12

The timing of the blood samples was as recorded in the legend to Table 37.

The shortening of the partial thromboplastin time in uterine vein blood during placental separation was highly significant ($R = 1$, $p<0.01$).

The data are graphically displayed in figure 17.

Table 32

One stage prothrombin time (seconds) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

	Uterine Blood				Peripheral Blood					Post Operation				
atient	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	11.0	11.0	11.0	12.0	11.0	11.0	12.0	11.5	13.0	14.5	14.0	14.0	14.0
2	14.5	12.0	11.5	13.0	14.5	14.0	14.0	13.5	13.0	13.0	-	13.5	13.0	15.0
3	-	12.0	14.0	12.0	14.0	14.5	14.0	-	13.5	14.0	13.0	15.5	23.5	20.0
4	-	-	-	11.5	-	12.0	11.0	11.0	11.5	-	13.0	14.0	12.5	14.0
5	14.0	13.0	13.0	14.0	14.0	13.0	13.0	12.0	12.5	13.0	13.5	14.0	13.0	12.5
6	14.5	-	-	13.0	13.0	-	13.0	-	15.0	14.0	11.0	-	-	-
7	13.0	13.5	13.0	-	13.5	14.0	-	-	13.0	-	-	14.5	-	-
8	11.0	10.5	11.0	10.5	12.0	11.5	12.0	12.0	-	11.5	11.5	-	12.0	11.5
9	13.0	-	12.5	13.0	12.5	-	12.5	12.5	-	13.0	12.0	11.5	14.0	12.0
10	10.0	10.0	10.0	10.0	10.0	10.0	10.0	11.0	-	12.0	14.0	13.0	12.0	-
11	15.0	13.5	14.5	14.5	15.0	14.0	14.5	13.5	15.0	14.0	15.0	13.0	14.0	15.0
12	14.0	13.0	14.0	13.0	14.0	14.0	13.0	13.0	-	13.0	12.0	14.0	12.5	-
ean	13.2	12.1	12.5	12.3	13.2	12.8	12.6	12.3	13.1	13.1	13.0	13.7	14.1	14.3
D.	1.7	1.3	1.5	1.4	1.4	1.6	1.4	0.9	1.4	0.8	1.3	1.1	3.4	2.7

The timing of the blood samples was as recorded in the legend to Table 37.

The data are graphically displayed in figure 17.

Table 33

Thrombin clotting time (seconds) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

Patient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	9.0	9.0	9.0	9.5	9.5	9.5	9.5	9.5	9.5	10.5	10.0	9.5	10.5
2	10.5	12.5	11.0	11.5	11.5	10.5	11.0	12.0	12.0	11.0	-	8.5	10.5	9.5
3	-	11.0	10.5	10.5	10.0	11.5	12.0	-	10.0	10.5	10.0	10.5	10.0	10.0
4	-	-	-	8.5	-	9.0	8.5	10.0	9.0	-	10.0	14.0	11.0	11.0
5	10.0	9.0	8.0	-	9.5	9.0	9.0	-	-	10.0	8.5	12.0	14.0	-
6	11.5	-	-	13.5	12.5	-	14.0	-	11.0	10.5	10.5	-	-	-
7	11.0	11.0	11.0	-	11.0	11.0	-	-	11.0	-	-	13.0	-	-
8	10.0	10.0	9.0	10.5	11.5	11.5	11.0	11.0	11.5	10.0	10.0	-	11.0	10.0
9	10.0	-	11.0	10.0	10.0	-	10.0	11.0	-	10.0	8.5	9.0	8.0	9.0
10	10.0	11.0	10.0	11.0	11.0	10.0	10.0	10.5	-	12.0	8.5	9.0	9.0	-
11	10.5	10.0	11.0	11.0	10.5	11.0	10.0	10.0	10.0	10.0	9.5	9.0	12.0	8.0
12	9.0	9.0	9.0	9.5	9.0	9.0	9.0	9.0	-	9.0	8.0	10.0	8.5	-
Mean	10.3	10.3	10.0	10.5	10.5	10.2	10.4	10.4	10.5	10.3	9.4	10.5	10.4	9.7
S.D.	0.7	1.2	1.1	1.4	1.1	1.0	1.6	1.0	1.0	0.8	0.9	1.9	1.8	1.0

The timing of the blood samples was as recorded in the legend to Table 37.

No significant change of the thrombin clotting time was detected in uterine blood or peripheral blood.

Table 34

Factor VIII(%) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	320	225	220	165	215	220	220	225	82	160	160	100	130
2	100	290	300	105	100	125	105	105	115	88	-	120	86	170
3	-	1500	450	1400	160	275	380	-	550	240	290	310	300	270
4	-	-	-	205	125	220	200	170	320	-	125	245	230	145
5	180	220	640	-	145	340	310	-	-	210	160	120	82	110
6	160	-	-	160	210	-	200	-	230	180	140	-	145	60
7	96	82	230	-	82	96	-	115	160	-	-	150	-	-
8	-	240	115	-	150	370	-	160	300	-	-	-	480	-
9	64	135	70	70	82	-	80	80	-	82	200	270	280	200
10	145	135	120	135	105	140	125	150	-	100	210	160	110	84
11	132	156	216	154	200	205	216	560	180	216	168	216	120	120
12	250	280	260	204	250	290	290	250	-	196	260	238	241	-
ean	141	336	263	297	148	228	213	201	260	155	190	199	197	143
D.	58	416	170	416	53	91	95	145	136	66	55	66	123	64

The timing of the blood samples for the factor VIII levels was as recorded in the legend to Table 37.

Factor VIII could not be assayed in two of the uterine vein samples taken during placental separation due to immediate clotting of the blood. The increase in factor VIII in the peripheral blood during placental separation is highly significant ($R = 0$, $p = 0.002$; Wilcoxon's Signed Ranks Test).

The data are graphically shown in figure 18.

Table 35

Factor IX(%) in uterine vein blood and peripheral vein blood
during placental separation and following delivery by caesarean section

Patient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	62	64	85	84	110	95	67	86	75	72	74	76	130
2	60	68	76	76	72	72	66	72	66	53	-	50	110	100
3	-	140	140	160	133	138	130	-	140	100	170	170	82	61
4	Not assayed													
5	105	62	94	-	110	160	125	-	105	160	140	-	110	100
6	62	-	120	-	78	75	-	80	-	130	280	-	130	120
7	80	-	-	130	80	80	-	86	120	120	-	120	-	100
8	47	-	42	40	50	42	47	-	-	80	110	120	70	120
9	105	90	85	75	110	140	120	100	120	-	140	120	110	100
10	74	96	80	80	100	110	110	80	80	100	180	160	130	120
11	105	94	80	76	120	110	110	110	76	80	135	140	-	-
12	Not assayed													
Mean	80	87	87	90	94	104	100	85	99	100	153	119	102	106
S.D.	23	28	29	37	25	36	30	15	26	33	61	41	24	20

The timing of the blood samples was as recorded in the legend to Table 37.

No significant change of the factor IX level was detected in uterine blood or peripheral blood.

The data are graphically displayed in figure 18.

Table 36

Factor V(%) in uterine vein blood and peripheral vein blood during placental separation and the levels following delivery by caesarean section

	Uterine Blood				Peripheral Blood					Post Operation				
atient	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	125	110	115	78	94	105	100	88	100	88	125	140	180
2	78	260	190	90	98	98	98	115	120	64	-	100	140	100
3	-	250	160	160	150	155	130	-	165	130	140	-	-	120
4	-	150	150	-	150	-	-	150	-	-	100	115	140	120
5	120	160	144	-	120	124	160	124	-	50	164	100	100	-
6	240	-	-	230	230	-	200	-	230	250	120	120	-	-
7	200	200	174	-	174	174	-	-	174	-	-	310	-	-
8	-	80	-	-	98	126	-	-	100	-	170	158	90	190
9	150	140	120	140	140	130	130	-	-	130	148	-	90	-
10	160	160	168	148	160	148	148	152	-	136	160	130	-	140
11	86	80	95	92	75	82	80	120	70	92	60	100	94	110
12	105	120	100	100	105	105	110	130	-	100	130	105	140	-
lean	142	156	131	134	132	124	129	127	135	117	128	136	117	137
.D.	57	57	45	47	45	29	37	19	57	58	36	64	25	35

The timing of the blood samples was as recorded in the legend to Table 37.

No significant change of the factor V level was detected in uterine blood or peripheral blood.

The data are graphically displayed in figure 18.

Table 37

Plasma fibrinogen (mg/ 100ml) in uterine vein blood and peripheral vein blood during placental separation and after caesarean section

Patient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	484	314	463	421	485	548	507	513	358	386	522	622	587
2	434	370	516	499	323	422	328	464	434	-	-	469	528	481
3	-	404	410	306	281	363	322	-	314	342	330	496	744	685
4	-	-	-	460	-	429	443	439	472	-	396	420	416	467
5	398	398	392	-	561	304	398	-	-	392	485	374	-	-
6	252	-	-	-	241	-	251	-	-	291	275	-	-	-
7	255	-	-	218	310	-	184	226	-	-	-	397	379	-
8	452	211	393	393	422	-	375	440	469	-	440	-	563	656
9	411	404	428	499	475	440	440	440	-	381	405	417	393	-
10	420	434	430	490	405	475	440	410	-	385	390	410	450	465
11	557	428	417	375	458	-	493	499	510	276	411	540	434	411
12	350	380	407	405	374	360	378	424	442	-	440	-	610	-
Mean	392	390	412	410	388	409	383	428	450	346	396	449	514	536
S.D.	96	75	52	92	94	62	102	82	67	46	59	59	120	106

Shown are the plasma fibrinogen levels in uterine vein blood draining the placental site and in peripheral vein blood taken simultaneously from the arm and the fibrinogen levels in peripheral blood following delivery by caesarean section. The specimens 'before delivery' were taken after exposure of the uterus and the 3 specimens under placental separation were (1) placenta separating 'in utero' (2) 5 minutes after placental separation (3) 15 minutes after placental separation.

The data are graphically shown in figure 21.

Table 38

Plasma plasminogen (casein units/ml) in uterine vein blood and peripheral vein blood during placental separation and after caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	3.3	3.4	3.3	3.5	3.8	3.5	3.4	3.4	3.2	3.6	3.6	3.4	3.8
2	4.1	3.7	3.2	3.7	3.8	3.8	3.9	4.0	3.8	-	-	2.9	3.4	-
3	-	3.5	3.1	3.2	3.9	3.8	3.0	-	3.4	3.4	3.0	2.3	2.7	2.2
4	-	-	-	3.2	-	4.4	3.2	3.1	-	3.3	3.2	3.0	3.9	3.9
5	3.7	3.6	3.5	-	4.1	4.0	4.2	-	-	-	3.7	4.5	-	3.9
6	4.3	-	3.9	-	4.2	3.9	-	3.9	4.1	-	4.9	-	-	-
7	4.8	5.3	5.3	-	4.7	4.3	-	-	4.6	-	-	4.3	-	-
8	-	4.9	-	-	4.9	5.0	-	-	5.1	-	-	-	-	-
9	3.3	-	2.9	2.8	3.2	-	3.3	3.1	-	2.9	-	3.3	3.0	3.4
10	3.4	3.5	3.6	3.6	3.7	3.5	3.5	3.6	-	3.2	3.1	3.6	-	3.9
11	3.1	3.3	3.2	3.3	3.2	3.3	3.2	3.2	3.1	3.0	3.1	2.8	3.0	3.5
12	3.8	4.0	3.9	3.8	4.3	4.0	4.0	4.1	3.9	4.0	3.4	-	3.5	-
ean	3.8	3.9	3.6	3.4	4.0	4.0	3.5	3.5	3.9	3.3	3.5	3.4	3.3	3.5
D.	0.6	0.7	0.7	0.3	0.6	0.5	0.4	0.4	0.7	0.4	0.6	0.7	0.4	0.6

The timing of the blood samples was as recorded in the legend to Table 37.

The data are graphically displayed in figure 21.

Table 39

Euglobulin lysis time (plasminogen activator) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	0.58	0.91	0.70	0.36	0.33	0.42	0.38	2.00	2.10	1.50	2.50	1.87	1.20
2	-	0.26	0.62	0.36	0.26	0.50	0.42	0.50	0.42	2.14	-	0.86	1.5	1.25
3	-	1.10	0.83	0.79	0.37	0.51	1.36	-	0.52	2.22	1.25	0.21	0.55	1.20
4	-	-	0.83	0.50	0.18	0.31	0.32	0.38	0.52	-	0.71	3.10	0.88	1.43
5	0.20	0.83	0.65	-	0.19	0.22	0.86	-	0.50	1.58	0.91	1.66	1.25	-
6	0.36	-	-	0.50	0.25	-	0.29	-	0.40	1.00	2.50	0.91	-	1.00
7	0.23	0.50	0.83	-	0.28	0.42	-	-	1.43	-	1.33	2.5	-	3.3
8	0.40	2.29	-	-	0.20	0.20	-	-	0.50	-	1.87	-	2.5	-
9	0.73	-	0.70	0.88	0.83	-	0.83	1.00	-	2.07	1.82	1.87	0.27	1.43
10	0.37	0.37	0.71	1.54	0.17	0.21	0.36	0.50	-	2.14	1.15	1.93	3.5	-
11	0.28	2.22	1.67	2.00	0.30	0.31	0.36	0.42	1.50	0.99	0.73	1.11	1.67	2.5
12	0.62	1.25	1.30	0.71	0.59	0.71	0.62	2.00	-	3.33	1.00	1.25	1.25	-
ean	0.40	1.04	0.91	0.89	0.33	0.37	0.58	0.74	0.87	1.95	1.34	1.63	1.52	1.66
D.	0.19	0.76	0.33	0.54	0.20	0.16	0.34	0.60	0.61	0.71	0.55	0.86	0.95	0.80

The timing of the blood samples was as recorded in the legend to Table 37.

The level of plasminogen activator in the uterine vein blood during placental separation was significantly higher than in the peripheral blood (R = 3, $p<0.05$) and a highly significant increase of plasminogen activator in the peripheral blood took place following delivery of the baby and separation of the placenta (R = 0, $p<0.001$).

The data are graphically shown in figure 22.

Table 40

Fibrin/fibrinogen degradation products (µg/ml serum) in uterine vein blood and peripheral vein blood during placental separation and following delivery by caesarean section

	Uterine Blood				Peripheral Blood					Post-Operation				
Patient	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	9.6	4.6	4.6	4.6	4.6	3.6	3.6	3.6	7.2	-	14.4	7.2	3.6
2	1.8	1.8	3.6	1.8	3.6	1.8	1.8	3.6	3.6	7.2	-	3.6	3.6	-
3	1.1	1.1	1.1	1.1	1.1	1.1	1.1	-	3.3	2.2	3.3	35.2	35.2	17.6
4	-	-	-	2.5	-	2.5	2.5	5.0	2.5	15.0	-	20.0	7.5	7.5
5	0.8	6.4	-	-	1.6	0.8	0.8	-	1.6	1.6	1.6	4.8	52.4	-
6	3.8	3.7	-	3.7	3.8	-	2.5	-	-	20.2	20.2	-	-	2.0
7	6.8	10.1	6.8	10.1	13.5	6.8	-	-	10.1	-	-	6.8	-	1.7
8	-	1.8	-	-	1.8	1.8	-	-	1.8	5.4	-	-	-	-
9	0.7	0.7	0.7	0.7	0.7	-	0.7	-	-	1.6	3.0	3.0	6.0	3.0
10	0.8	0.8	1.5	1.5	1.5	1.5	1.5	1.5	-	3.0	3.0	1.5	1.5	-
11	3.0	1.5	1.5	3.0	1.5	1.5	1.5	1.5	3.0	1.5	3.0	2.8	2.8	2.4
12	2.4	2.4	2.4	2.4	4.7	3.5	2.3	2.3	2.3	4.7	1.7	2.3	4.7	-
Mean	2.35	3.62	2.78	3.14	3.49	2.59	1.83	2.91	3.53	6.33	5.11	9.44	13.43	5.40
S.D.	1.99	3.47	2.09	2.71	3.61	1.88	0.90	1.39	2.57	6.07	6.69	10.86	17.85	5.72

The timing of the blood samples was as recorded in the legend to Table 37.

The data are graphically displayed in figure 22.

Table 41

Urokinase sensitivity test (units) in uterine vein blood and peripheral vein blood during placental separation and after caesarean section

atient	Uterine Blood				Peripheral Blood					Post Operation				
	Before delivery	Placental separation (1)	(2)	(3)	Before delivery	Placental separation (1)	(2)	(3)	End of operation	2-4 hours	24-48 hours	3-5 days	6-9 days	10-14 days
1	-	0.80	0.77	0.82	1.18	1.19	1.04	0.76	0.89	0.82	0.56	0.72	0.65	0.68
2	0.77	0.51	0.47	0.49	0.62	0.58	0.65	0.66	0.67	-	-	0.56	0.69	0.55
3	-	1.25	1.13	0.90	1.24	1.31	1.22	-	1.04	1.40	1.03	0.25	0.25	0.65
4	-	-	-	1.37	-	1.34	1.07	1.08	0.98	-	0.50	0.49	0.54	1.89
5	0.58	0.77	0.54	-	0.58	0.53	0.54	0.64	-	0.65	0.82	-	0.62	-
6	1.24	-	-	1.01	1.64	-	1.26	-	1.08	1.01	0.71	-	-	-
7	1.82	1.43	1.47	-	0.91	1.47	-	-	1.40	-	-	1.61	-	-
8	-	-	-	-	2.90	2.70	-	-	2.80	-	-	-	-	-
9	0.11	0.11	0.34	0.18	0.32	-	0.32	0.39	-	0.45	0.29	0.68	0.33	0.53
10	0.55	0.55	0.74	1.24	0.62	0.63	0.55	0.57	-	0.86	0.87	0.62	-	0.62
11	0.54	0.57	0.54	0.44	0.53	0.51	0.44	0.41	0.42	0.42	-	1.04	0.48	0.68
12	0.44	0.70	0.87	0.85	0.76	0.72	0.80	0.33	-	1.01	0.77	0.58	0.54	-
ean	0.76	0.74	0.76	0.81	1.02	1.10	0.79	0.60	1.16	0.83	0.69	0.73	0.51	0.80
D.	0.53	0.40	0.36	0.38	0.73	0.68	0.34	0.24	0.72	0.32	0.23	0.39	0.15	0.48

The timing of the blood samples was as recorded in the legend to Table 37.

No significant change in the levels of inhibitor to urokinase induced lysis was detected in uterine blood or peripheral blood.

The data are graphically displayed in figure 22.

Table 42

Coagulation tests in the healthy new born and in the mother immediately after birth

	Recalcification time in plastic (minutes)		Kaolin cephalin clotting time (seconds)		Partial thrombo- plastin time (seconds)		Thrombin clotting time (seconds)		Prothrombin time one stage (seconds)	
	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Maternal
1	5.3	6.7	63	47	73	62	11.0	9.5	17.0	12.5
2	5.7	5.2	54	39	88	69	12.5	11.5	23.0	13.0
3	5.9	4.0	60	45	71	60	11.5	10.0	16.0	11.0
4	5.1	6.2	75	41	109	60	11.0	10.0	17.0	10.5
5	3.9	5.6	48	38	66	47	11.5	10.0	15.0	13.0
6	5.0	5.8	51	30	87	57	11.5	10.5	16.0	13.0
7	4.2	7.9	55	42	61	53	12.0	10.5	14.5	12.0
8	4.6	5.4	47	49	56	48	10.5	10.0	16.0	14.0
9	5.8	5.9	63	45	59	59	11.0	9.0	20.0	12.0
10	4.6	4.8	66	40	105	58	12.0	10.0	18.0	12.0
Mean	5.0	5.8	58	42	78	57	11.5	10.1	17.3	12.3
S.D.	0.7	1.1	9	5	19	7	0.6	0.7	2.6	1.0
R	12		1		0		0		0	
p	NS		<0.01		0.002		0.002		0.002	

With the exception of the recalcification time in plastic, the coagulation tests in the newborn were significantly longer than the maternal values.

Table 45 A

The coagulation factors and platelet count in the newborn and in the mother immediately after birth

	Factor II%		Factor V%		Factor VIII%		Factor IX%		Factor X%		Platelets (x1000/ cmm)	
	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother
1	44	100	70	66	142	170	14	84	30	170	388	185
2	28	135	96	70	198	198	22	94	17	115	305	208
3	48	115	-	100	311	308	26	120	47	115	281	267
4	46	150	120	85	110	137	10	120	50	250	284	192
5	65	190	100	100	226	226	24	110	55	110	259	184
6	65	150	170	160	504	528	10	78	58	170	323	268
7	80	180	300	205	135	220	10	100	55	180	319	189
8	82	130	290	180	280	175	23	74	31	120	390	308
9	32	135	88	88	170	240	21	140	29	220	259	217
10	47	160	270	210	280	400	7	76	22	220	206	260
ean	54	145	167	121	236	260	17	100	39	167	301	228
D.	19	28	94	53	116	121	7	22	15	51	57	44
R	0		0		7		0		0		2.5	
p	0.002		<0.05		NS		0.002		0.002		<0.01	
orrelation oefficient (r)	0.433		0.955		0.868		0.363		0.012		0.109	
p	NS		<0.001		<0.01		NS		NS		NS	

Factors II (prothrombin), IX and X were markedly lower in the newborn than in the mother and no significant correlation was present between the respective levels in the mother and baby. No significant difference was present between the level of factor VIII in the newborn and the mother but factor V was significantly higher in the newborn. A significant correlation was present between the levels for factor V and VIII in the mother and baby. The platelet count was significantly higher in the baby than the mother but no correlation was present between the maternal and neonatal levels.

The data are graphically displayed in figure 24.

Table 43 B

Factor VII in the newborn and the mother immediately after birth

	Newborn	Mother
1	42	140
2	53	180
3	50	350
4	17	370
5	45	200
6	21	210
Mean	38	242
S.D.	15	95
t	5.187	
p	<0.01	

Factor VII was assayed by using plasma with a congenital deficiency of this factor. The assay was performed in only six mothers and their babies owing to insufficient test plasma in the others. The factor VII levels were low in the newborn in contrast to the increased levels in the mother. No correlation was present between the levels in the mother and baby.

Table 44

The findings in the fibrinolytic enzyme system in the newborn and in the mother immediately after birth

	Fibrinogen (mg/ 100ml)		Plasminogen (casein units/ ml)		Euglobulin lysis activity (units)		Urokinase sensitivity (units)		Fibrin degradation products (µg/ ml serum)	
	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother	Newborn	Mother
1	215	372	1.2	2.95	20.0	0.30	0.08	0.66	1.4	5.6
2	187	363	1.3	2.2	4.5	0.25	0.19	0.74	4.0	4.0
3	164	334	-	2.65	4.0	0.20	0.04	0.50	1.9	3.8
4	226	515	1.4	2.7	15.0	0.33	0.11	0.87	1.4	1.4
5	231	311	1.4	2.2	12.0	0.63	0.18	0.87	2.4	1.2
6	214	434	1.3	3.1	4.6	0.36	0.12	0.77	2.0	2.0
7	180	270	1.1	2.3	10.0	0.36	0.11	0.95	1.4	5.6
8	199	374	1.2	3.4	10.0	0.50	0.15	1.18	2.0	2.0
9	137	380	1.0	2.9	4.3	0.31	0.14	0.78	1.1	1.1
10	133	282	1.4	2.6	5.0	0.62	0.08	0.76	1.7	6.8
Mean	189	363	1.3	2.7	8.9	0.39	0.12	0.81	1.9	3.4
S.D.	35	73	0.1	0.4	5.5	0.15	0.05	0.18	0.8	2.1
p	<0.002		<0.002		<0.002		<0.002		<0.05	

The fibrinogen and plasminogen levels were significantly lower in the newborn than in the mother. However, no significant correlation was present between the maternal and neonatal levels. Euglobulin lysis activity and urokinase inhibitor were both considerably higher in the newborn than in the mother but no significant correlation was present between these tests in the baby or the mother. Fibrin/fibrinogen degradation products were significantly lower in the newborn than in the mother.

The data are graphically displayed in figure 25.

TABLE 45.

The levels of fibrin degradation products (F. D. P.) and plasminogen in peripheral blood and blood escaping from the uterus or in proximity to the retroplacental clot in abruptio placentae in the same patients. Uterine blood levels of F. D. P. are significantly less than peripheral blood ($R = 0$, $p < 0.01$).

F. D. P. (μg/ml)		Plasminogen (casein units/ml)	
Peripheral blood	Uterine blood	Peripheral blood	Uterine blood
690	86	2.5	2.2
435	54	3.0	3.5
650	82	2.8	3.0
614	460	2.1	2.3
1220	614	1.2	1.1
652	538	1.3	1.35
1280	640	1.2	1.1
480	480	3.7	1.3
752 ± 320	369 ± 252	2.2 ± 0.9	2.0 ± 0.9

TABLE 46

Fibrinogen, plasminogen and serum fibrin/fibrinogen degradation products (FDP) in patients with severe pre-eclampsia and eclampsia. The control values were those obtained from healthy pregnant women matched for age and gestation.

	Fibrinogen (mg/100 ml.		Plasminogen (Casein Units/ml)		Serum F. D. P. (μg/ml)	
	Patients	Controls	Patients	Controls	Patients	Controls
	380	298	2. 8	3. 4	4. 4	4. 0
	342	400	2. 6	3. 2	5. 6	1. 0
	322	358	3. 0	3. 3	2. 2	1. 7
	278	450	2. 5	4. 1	1. 8	4. 0
	312	380	3. 5	3. 2	26. 4	1. 0
	352	345	2. 4	2. 6	12. 0	1. 0
	336	370	2. 6	4. 1	10. 2	2. 2
	472	340	3. 2	2. 7	10. 0	1. 0
	270	304	2. 6	2. 8	13. 2	2. 2
	505	440	4. 2	4. 5	3. 6	1. 0
Mean	357	369	2. 9	3. 4	8. 9	1. 9
S. D.	77	51	0. 6	0. 7	7. 4	1. 2
	$t = 0.397$		$t = 1.522$		$t = 2.968$	
	N. S.		N. S.		$p < 0.01$	

Table 47

Euglobulin lysis activity (units) and urokinase sensitivity (units) in patients with severe pre-eclampsia and eclampsia. The control values were those obtained from healthy pregnant women matched for age and gestation.

	Euglobulin Lysis Activity (units)		Urokinase Sensitivity (units)	
	Patients	Controls	Patients	Controls
	0.185	0.250	0.670	0.667
	0.227	0.400	0.500	0.667
	0.167	0.333	0.392	0.714
	0.178	0.333	0.200	1.176
	0.208	0.417	0.417	0.752
	0.178	0.333	1.408	1.176
	0.152	0.556	0.667	0.752
	0.238	0.200	0.971	1.250
	0.250	0.313	0.323	1.333
	0.208	0.240	0.667	1.667
Mean	0.199	0.338	0.622	1.015
S.D.	0.030	0.102	0.353	0.350
	$t = 4.144$		$t = 2.507$	
	$p<0.01$		$p<0.05$	

Table 48

Factor VIII activity and the platelet count in patients with severe pre-eclampsia and eclampsia. The control values were those obtained from healthy pregnant women matched for age and gestation.

	Factor VIII(%)		Platelets (X1000 per c. mm)	
	Patients	Controls	Patients	Controls
	156	140	155	230
	180	205	144	200
	150	200	176	175
	184	260	162	195
	310	235	118	262
	100	175	88	254
	94	230	178	180
	300	116	85	198
	85	145	192	176
	335	138	100	160
Mean	189	184	140	203
S.D.	94	49	39	35

t = 0.150
N.S.

t = 3.809
$p < 0.01$

APPENDIX 3

METHODS

APPENDIX 3

METHODS

In this section the laboratory methods used in the thesis are described. Wilcoxon's Signed Rank Test which was used to determine the significance of group changes is also explained.

Laboratory Methods

Collection of Samples. For coagulation and fibrinolytic assays the blood was obtained by clean venepuncture using plastic syringes and 9ml of blood was added to 1ml of 3.8 per cent sodium citrate in a 13 x 90mm plastic tube (Luckam Ltd., Kingston Road, Raynes Park, London). The tubes were kept on melting ice and the plasma separated by centrifugation (600g for 10 minutes at 4°C). The assays were performed within 30 minutes of the venepuncture and no correction of the results was made for dilution by citrate.

Coagulation System Assays:

Whole Blood Clotting Time. Clotting times were performed in plastic tubes by a modification of the method of Lee and

White (1913). A minimum of 10ml of blood was drawn by clean venepuncture and the needle removed from the plastic syringe. One ml of blood was added immediately to two clean dry plastic tubes (13 x 90mm) which were observed for clotting in a water bath at 37°C. The first tube was tilted once a minute until clotting occurred. The second tube was left undisturbed until the first tube clotted and was then tilted at one minute intervals until clotting took place. The end point was taken as the time when no blood ran down the side of the tube on tilting and the time that the second tube clotted was recorded as the whole blood clotting time.

Plasma Recalcification Time. This test was performed by mixing 0.1ml aliquots of non-contacted plasma and 0.9 per cent saline at 37°C in 65 x 10mm plastic tubes (Luckam Ltd., Kingston Road, Raynes Park, London). The time taken for clotting to occur after the addition of 0.1ml of 0.025M calcium chloride was recorded. Each test was done in duplicate and a normal control was included in each batch of tests.

Kaolin Cephalin Clotting Time. The method used was based on that of Proctor and Rapaport (1961). The test depends on the level of fibrinogen, factors II, V, VIII, IX, X, XI and XII. Elevated levels of one or more individual factors or the presence of active intermediates tend to reduce the clotting time. Minor deficiencies in several factors may on the other hand result in prolongation of the clotting time. 0.1ml of plasma was activated with 0.1ml of platelet substitute (Parke Davis) and 0.1ml of kaolin (5mg/ml saline) for 10 minutes at 37°C. After the addition of 0.1ml 0.025M calcium chloride the clotting time was recorded. Each test was done in duplicate and a normal control included with each batch of tests.

Partial Thromboplastin Time. The method used was that of Langdell et al (1953). This test is similar to that of the kaolin cephalin clotting time except that the plasma is not maximally contacted by kaolin. 0.1ml of plasma was mixed with 0.1ml of serum, 0.1ml of platelet substitute and 0.1ml of 0.025M calcium chloride. A stop-watch was started on the addition of the calcium chloride and the

clotting time recorded. Each test was done in duplicate and a normal control included with each batch of tests.

One Stage Prothrombin Time. This test was carried out at 37°C as described by Douglas (1962). 0.1ml of the citrated plasma was mixed with 0.1ml of a saline extract of human brain and 0.1ml of 0.025M calcium chloride and the clotting time recorded. The tissue extract and calcium are present in optimum amounts and the clotting time reflects the amount and speed of thrombin formation and the reactivity of the fibrinogen.

Thrombin Clotting Time. The method used was that described by McNicol and Douglas (1964). To 0.1ml of plasma was added 0.3ml of 'thrombin titration mixture' (Seegers and Smith, 1942). Thrombin, 0.1ml (6 N.I.H. units/ml) was added and the clotting time of the mixture recorded at 37°C. The thrombin titration mixture was prepared immediately before use by mixing 6ml of 0.9 per cent saline, 2ml of 0.7 per cent calcium chloride, 2ml of 15 per cent acacia and 1ml tris buffer, 0.1M, pH 7.5. The test is used as an index of defective fibrin polymerisation.

Factor II (Prothrombin) Assay. A one stage method was used based on that described by Owren and Aas (1951). This test was designed so that the only variable which would determine the prothrombin time was the concentration of factor II. Fibrinogen and factor V were provided by bovine plasma from which factors II and VII had been removed by absorption on barium sulphate, and human serum was included to restore the factor VII removed by absorption. Using silicone coated pipettes 0.1ml of a tenfold dilution of the plasma was added to 0.1ml of the human serum-bovine plasma mixture in a pyrex tube; 0.1ml of a thromboplastin calcium chloride mixture was then added and the stop-watch started. The tube was tilted continually until a clot forms. A normal fresh plasma was used as a control and the value obtained was taken as equivalent to 100 per cent prothrombin concentration. Serial dilutions were performed and the dilution graph was prepared on logarithmic paper. The prothrombin content of the test sample was calculated by interpolating the clotting time on the dilution curve of the normal plasma.

Factor V Assay. The method used was based on that of Shanberge et al. (1967). Factor V deficient plasma was made by collecting normal blood into oxalate and storing the plasma at 4°C for 1-2 weeks. Normal fresh plasma which was assumed to contain 100 per cent factor V activity was serially diluted with Owren's buffer pH 7.3. 0.1ml of the plasma was added to 0.1ml of factor V deficient plasma at 37°C. 0.2ml of a mixture of saline extract of brain and 0.025M calcium chloride was added to the plasma and the clotting time recorded. Each dilution was tested in duplicate and a graph prepared on logarithmic paper. The test plasma was measured for factor V activity in the same system.

Factors VIII and IX Assays. These were carried out by a one stage test according to the method of Breckenridge and Ratnoff (1962) which depends on the clotting time of substrate plasma from patients with congenital deficiency of factor VIII and factor IX. Kaolin 50mg was mixed with a 1 in 4 dilution of platelet substitute and 0.1ml of the homogenised mixture was added to as many tubes as required up

to a maximum of 6 tubes. 0.1ml of substrate plasma was then added and the tube incubated for 10 minutes at 37°C. After 8 minutes a 1 in 10 and 1 in 100 dilution of the test plasma in Owren's buffer was prepared on melting ice. At 10 minutes 0.1ml of the diluted plasma was transferred to the incubation tube and 0.1ml of 0.025M calcium chloride added. After mixing the tubes were left at 37°C before tilting continually until clotting occurred. Clotting times were determined in duplicate for dilutions of both test and control plasmas. The curve of the clotting time against plasma concentration was plotted on semi-logarithmic paper and the concentration of the test plasma was obtained by interpolation.

Factor X Assay. The method used was that of Denson (1961). Factor X substrate plasma and Russell's Viper Venom (RVV) cephalin reagent (Diagnostic Reagents, Oxford) were used. 0.1ml of a 1 in 10 dilution of the test plasma in Michaelis buffer pH 7.3 was added to 0.1ml of the substrate plasma. 0.1ml of the RVV in cephalin was then added. After 30 seconds 0.1ml of 0.25M calcium chloride was added and the

clotting time recorded. A calibration curve was constructed on logarithmic paper by similarly testing dilutions of normal plasma. The assays were performed in duplicate and the test plasma was expressed as a percentage of the control plasma.

Fibrinolytic Enzyme System Assays:

Fibrinogen Assay. The method used was that of Ratnoff and Menzie (1965). In this assay fibrinogen is clotted with thrombin in the presence of crushed pyrex glass; the fibrin so formed is hydrolysed with sodium hydroxide and the tyrosine released is estimated calorimetrically. A constant proportion of tyrosine in the fibrinogen molecule is assumed (1:11.7).

Assay Technique. To a 40ml pyrex centrifuge tube was added "0.5ml" of crushed glass, 10ml of 0.9 per cent saline and 0.05ml of thrombin (Parke-Davis, 1000 N.I.H. units/ml). 0.5ml of the test plasma was pipetted into the tube which was then thoroughly agitated on a Whirlimixer (Fisons, Loughborough, England). After leaving the mixture for 10 minutes at 4°C the mixture was centrifuged for five minutes at 500g. The supernatant was discarded and the crushed glass with adherent fibrin was washed twice with 10ml of saline. After final

centrifugation and decantation of the washing fluid, 1ml of 10 per cent sodium hydroxide solution was added and the tube was heated in a boiling water bath for 10 minutes. Evaporation was minimised by covering the tube with an aluminium cap. After cooling to room temperature, 7ml of water was added followed by 3ml of 20 per cent sodium carbonate solution and 1ml of Folin-Ciocalteu. The mixture was agitated on a Whirlimixer. After standing for 10 minutes to allow for colour development, the optical density at 650mμ was read against a reagent blank. Standard tyrosine solutions were prepared and the amount of fibrinogen in the test plasma was calculated by comparing its optical density with that of the standard tyrosine solutions.

Plasminogen Assay. The method used was the caseinolytic assay of Remmert and Cohen (1949) as modified by Alkjaersig et al (1959) and described by McNicol and Douglas (1964). Antiplasmin is first destroyed by incubating the plasma with acid. The acid is neutralised with alkali and buffer, and streptokinase is then added to convert the plasminogen to plasmin. The plasmin so produced is assayed by a caseinolytic technique, the amount of tyrosine released from

the casein being a measure of the amount of plasmin present. For the plasminogen assays in the studies reported in Chapters 4 and 10 the casein employed was obtained from Light and Co. Ltd., Colinbrook. Subsequent supplies of casein from this source proved unsatisfactory and 'Hammarsten' casein, British Drug Houses, was employed for the other investigations. The plasminogen results in Chapters 4 and 10 are thus not strictly comparable with the results in the other investigations. The casein solution was prepared by boiling for 20 minutes 25gm of casein in 500ml phosphate buffer 0.1M, pH 7.6. The solution was filtered while hot and after cooling the pH was readjusted to 7.6. The solution was then dialysed overnight against a large volume of constantly stirred phosphate buffer.

Assay Technique. To 0.5ml plasma was added 0.5ml 1/6N hydrochloric acid. After standing for 15 minutes at room temperature to destroy antiplasmin, 0.5ml 1/6N sodium hydroxide was added, followed by 1.0ml phosphate buffer, 0.1M, pH 7.6, 0.5ml streptokinase solution 2000units/ml (Varidase, Lederle) and 2.0ml 5 per cent casein solution.

After addition of casein and thorough mixing, the assay mixture was incubated at 37°C for 62 minutes. At 2 minutes and 62 minutes, 2ml aliquots were taken and to each was added 2ml 10 per cent trichloracetic acid. After centrifugation (500g for 10 minutes) 1ml of the supernatant was added to 5ml 0.5N sodium hydroxide and 1.5ml 5 per cent trichloracetic acid, followed by 1.5ml dilute (1:2) Folin-Ciocalteu reagent. After standing for 15 minutes for colour development, the optical density of the 62 minute sample was read at 650mμ with the 2 minute sample as blank. Tyrosine release was read off a standard curve; 1 casein unit equals 180μg tyrosine released in 1 hour.

Euglobulin Lysis Activity. In this test, the plasma euglobulin fraction is precipitated at pH 5.4 and low ionic strength. The precipitate contains plasminogen activator and plasminogen, a quarter of the plasma fibrinogen, but with only traces of the plasma antiplasmins which are chiefly in the supernatant (Kowalski et al, 1959). Because antiplasmin is not present in the clots made from the euglobulin precipitate, lysis times are much shorter than those found with clots made from

native plasma. In the presence of normal concentrations of plasma plasminogen and fibrinogen, the lysis time is a measure of the plasma activator activity. Low plasminogen concentrations, even in the presence of high activator activity, lead to prolonged euglobulin lysis times because there is insufficient potential plasmin to digest the fibrin of the clot (McNicol et al, 1962).

The technique used was that of Nilsson and Olow (1962). To 9.5ml of 0.014 per cent acetic acid, pH 5.4, was added 0.5ml plasma. The mixture was kept at 4°C for 10 minutes and then centrifuged at 4°C at 500g for 10 minutes. The supernatant was discarded and the precipitate dissolved in 0.5ml of 0.1M barbitone buffer, pH 7.3 (sodium diethyl barbitone, 11.75gm; HCl, 0.1N, 430ml; Na Cl, 14.67gm; distilled water to 1000ml). After clotting with thrombin, 0.5ml of a 2 N.I.H. unit/ml solution, the clot was incubated at 37°C and the time necessary for complete clot lysis was recorded. In the sequential investigation of the fibrinolytic changes during and after normal childbirth and in the uterine circulation during caesarean section, clot lysis was measured by automatic recording of the optical density of the clot (Cash

and Leask, 1967).

Sherry and Alkjaersig (1957) have shown that in fibrinolytic assays, activity is proportional to the reciprocal of the lysis time and accordingly a logarithmic plot of lysis time against units of activity shows a linear relationship. Such a plot can be used to express observed lysis times in terms of arbitrary units of activity, as suggested by Sherry et al (1959). Such units can be used for statistical purposes in analysis of data. In the present studies, a lysis time of 300 minutes was assigned 1 unit of activity. Observed lysis times can be converted to units by dividing the lysis time in minutes into 300.

Urokinase Sensitivity Test. This test was carried out as described by McNicol et al (1963) as an assay of fibrinolytic inhibitor activity. The plasma is clotted in the presence of a standard amount of the plasminogen activator, urokinase, and in any one patient in whom the fibrinogen and plasminogen levels are relatively constant, changes in lysis times will provide a comparative measure of overall fibrinolytic inhibitor levels. The test does not distinguish between anti-activator and antiplasmin activity, although with the relatively

high concentration of urokinase used in the test system variations in lysis times are more likely to be due to changes in antiplasmin activity. In this assay 0.32ml of urokinase (500 Ploug. units/ml) was added to 0.2ml of plasma. The mixture was clotted with 0.1ml of thrombin (20 N.I.H. units/ml). The lysis time of the plasma clot at 37°C was measured. The results can be expressed in minutes but for statistical analysis units of activity are used, one unit being arbitrarily defined as the reciprocal of a lysis time of 10 minutes.

Assay of Fibrin/Fibrinogen Degradation Products

Fibrin/fibrinogen degradation products (FDP) were assayed on a haemagglutination inhibition system which was a modification of the methods described by Murakami et al (1965) and Merskey et al (1966). The basic reagents in the assay are fibrinogen-coated red cells, anti-fibrinogen serum and the test serum sample. The unknown serum sample is first serially diluted and incubated with the antiserum which is then bound proportionately to the concentration of antigenic fibrinogen material in the sample. If no FDP are present in the diluted sample the mixtures

will retain the full amount of fibrinogen antibody; the more FDP present in the serum the lower the residual antibody that will remain in the incubation mixture. After incubation the unbound fraction of the antiserum is quantitated by adding a suspension of tanned formalinised fibrinogen-coated red cells. The level of antigenic material in the unknown serum sample can be estimated from the dilution of the serum which inhibits the agglutination of the coated red cells. The more the residual antibody in the serum mixtures the lower the titre of inhibition of red cell agglutination, and similarly the higher the titre of inhibition the higher the concentration of FDP in the unknown sample. The sensitivity of the system is calculated by assaying a standard solution of fibrinogen or pooled plasma. The microtitre system (Flow Laboratories Ltd., Irvine, Scotland) facilitates the use of intermediate dilutions to increase the precision of assay.

Preparation of Sensitised Sheep Cells. Fresh sheep red blood cells were washed three times in 50 volumes of sterile saline, the cells being spun at 1000r.p.m. One volume of

8 per cent sheep red cells in saline was then mixed with an equal volume of 3 per cent buffered formalin pH 7.2 and the cells agitated gently for 18 hours at 37°C with a magnetic stirrer. The formalinised cells were next passed through the filter of an intravenous infusion set to remove any adherent clumps of cells. The cells were again washed three times with 50 volumes of phosphate buffer pH 6.4. The formalinised cells were stored at 4°C in a 2 per cent suspension in phosphate buffer. Tannic acid (10mg/ml water) was diluted 1 in 400 with phosphate buffer and equal volumes of this freshly prepared solution and 2 per cent formalinised cells were mixed and incubated at 56°C in a water bath for 1 hour with gentle stirring. The tanned cells were then washed three times with 50 volumes of citrate buffer pH 6.4 and made up to a 4 per cent suspension in citrate buffer. The cells were sensitised with fibrinogen by mixing equal volumes of the tanned formalinised cells and a solution of Kabi purified human fibrinogen (20μg/ml) at 37°C for one hour. The sensitised cells were then washed three times at 4°C with 50 volumes of citrate buffer pH 6.4. The diluent for storing the red cells and for the test system was

made up in 500ml quantities and consisted of autoclaved citrate buffer pH 6.4 containing 0.25 per cent horse serum (Welcome No. 5 Horse Serum) adsorbed with sheep red cells, 0.01 per cent sodium azide and 0.02 per cent tranexamic acid. The sensitised cells were stored in 3ml aliquots at 4°C as a 2.5 per cent suspension in diluent, this quantity providing sufficient cells for one microtitre plate.

Collection of Blood Samples for FDP Assay. To inhibit any 'in vitro' fibrinolytic activity after collection, the blood specimens (5ml) were added to tubes containing 0.05ml of tranexamic acid (20mg/ml); glass beads were also present in the tube to promote clotting. The specimens were incubated for at least two hours in a water bath at 37°C and the serum was then removed after centrifugation. The serum samples were stored at -20°C until assayed. In patients who had a prolonged thrombin clotting time the test serum was incubated for two hours at 37°C with about 1/10 of its volume of thrombin (100 units/ml) to remove any residual thrombin clottable fibrinogen. In the test samples where raised levels were found, the assay was repeated after further incubation with thrombin, so that as far as was

possible only immunological reactive products which were not clottable by thrombin were measured.

Assay Procedure. The test serum was adsorbed for two hours at 4°C with 1/5 volume of packed fresh sheep red cells to remove any anti-species agglutinins. The mixture of cells and serum was then centrifuged and the clear serum assayed immediately for FDP using the microtitre system (Cooke Engineering Co., California). Serial dilutions of the test serum were made in microtitre plates (13cm x 8.3cm) with 'V' shaped wells. Rabbit anti-human fibrinogen serum (Hoechst) in a dilution of 1/4000 to 1/10,000 was added and the plates left at 4°C for one hour. Freshly suspended sensitised cells were added to the plates which were sealed, gently agitated and left at 4°C overnight before the results were read. Positive and negative controls were included with each test serum and, to determine the sensitivity of the system, three concentrations of a fibrinogen standard, either a solution of purified human fibrinogen (Kabi) or a standard pooled plasma were included in each batch of assays. The end point was taken as the last dilution showing a button or ring pattern of cells with no trace of agglutination. The sensitivity of the FDP assay was usually in the range 0.6-1.5μg/ml.

Tests of Platelet Function:

Platelet Count. Blood for platelet counting was collected into polystyrene tubes containing EDTA. 0.1ml of blood was added to 10ml of freshly filtered formol citrate diluting fluid in a clean test tube (Dacie, 1963). The contents of the tube were mixed by inversion 20 times and an improved Neubauer counting chamber was filled. The chamber was left for 20 minutes in a humid atmosphere before a direct platelet count was performed.

Platelet Adhesiveness. The method of Hellem (1960) as modified by Hirsh et al (1966) was used. Glass bead columns were made by filling a length of transparent vinyl plastic tubing (Portland Plastics Ltd., Kent) with 2.5g of Ballotini glass beads (0.57mm diameter) to give a column 6cm in length. Blood was collected into a plastic syringe and mixed with 3.8 per cent sodium citrate (nine volumes of blood to one volume of citrate) in a siliconised graduated centrifuge tube using parafilm over the end of the tube. The blood was left at room temperature for 30 to 45 minutes and then mixed by gentle inversion 20 times. 5ml of the blood was added to a polystyrene tube and 2ml drawn into a 2ml graduated plastic

syringe. The syringe was fitted to one end of the glass bead column and the blood was forced through the column at a constant rate by an electrically driven mechanical pump. The blood issuing from the other end of the column was collected in a polystyrene tube. The two blood samples, before and after passage through the column, were mixed 10 times by inversion and duplicate counts performed on each sample. The difference between the two counts was expressed as a percentage of the initial platelet count and this value was taken as an index of platelet adhesiveness in the sample. Appropriate corrections were made depending on the packed cell volume of the sample (Hellem, 1960).

Platelet Aggregation. The turbidimetric method described by O'Brien (1962) and Born and Cross (1963) was used to measure platelet aggregation by recording the changes in optical density occurring as a result of the addition of 0.1ml of adenosine diphosphate (0.25μg/ml) to 1.9ml of platelet rich plasma. The apparatus consisted of an EEL titrator connected to a galvanometer. A perspex cuvette was fitted onto the titrator above a magnetic stirrer in the light path from the photo-electric cell. The changes of optical density

after addition of the aggregating agent were recorded every 30 seconds for 10 minutes at room temperature. The maximum aggregation was measured by subtracting the lowest optical density recorded from the optical density before the addition of ADP.

Statistical Tests

For analysis of the data in the investigations recorded in this thesis Student's t test was used for comparing different groups of results and where an apparent relationship between results of different tests was found the correlation coefficient and equation of the regression line was calculated.

Wilcoxon's Signed Ranks Test (1945) was used to analyse the findings when a single group was examined at different stages. The test depends on the fact that if no significant difference exists between two sets of paired measurements, any chance differences which are present ought to consist of about equal numbers of plus and minus differences. The rank values are summed as plus or minus values and the smaller rank total (R) determines the significance of any difference between the groups of

measurements. The probability levels are obtained by reference to a table of R values relative to the number of the paired measurements (Langley, 1968).