1966

Coagulation alterations of normal pregnancy : a review of the recent literature and study of a series of pregnancy women late in the 3rd trimester of pregnancy

Klemens Elden Gustafson
University of Nebraska Medical Center

COAGULATION ALTERATIONS OF NORMAL PREGNANCY: A REVIEW OF THE RECENT LITERATURE AND STUDY OF A SERIES OF PREGNANT WOMEN LATE IN THE THIRD TRIMESTER OF PREGNANCY

Klemens Elden Gustafson

Submitted in Partial Fulfillment for the Degree of
Doctor of Medicine

College of Medicine, University of Nebraska

February 1, 1966

Omaha, Nebraska

TABLE OF CONTENTS

Through the years the pregnant state and parturition have proven a source of fascination for the scientist and philosopher both medical and nonmedical. With the implementation of scientific discoveries this period has gradually been reduced to a well ordered series of steps, stages, phases, and periods. Phenomenal discoveries have shed light upon its once mystery shrouded processes until the scientist is now able to compete successfully with the philosopher in explaining the majority of the events occurring during this period. This has come about in no small measure through the ability to document physiologic alterations in a scholarly manner with measurements of complex hormones, blood, tissue fluids, and their contents. Within this spectrum of scientific advances has proceeded a more nearly mature understanding of a separately mysterious and philosophical process of old, namely, the coagulation of blood. A dual fascination for these processes forms the basis for the study at hand. Many alterations in the coagulation system have been recognized during the course of pregnancy; yet with the very recent acquisition of knowledge on the subject, the specific nature of these changes has not been made completely clear.

It was felt that through a review of the recent literature on the subject an insight might be acquired into the nature of these phenomena, and through the study of a series of normal pregnant women late in the third trimester of pregnancy some insight might also be gained into the integrated effect(s) of

these changes. Therefore, an attempt was made to perform a rather extensive review of the medical literature pertinent to the coagulation system during normal pregnancy concentrating on that published since 1960. As always much of the older material published in the field is of such consequence as to demand inclusion in this review. At the end it is hoped that the paper may provide a current reference to the more significant contributions in this area of endeavor.

Although it is appreciated that the entire concept of hemostasis is quite broad including such phenomena as vessel contractility, hemodynamics of the blood vascular system, platelet activity, and others, only those factors of primary concern to blood coagulation shall be considered at this time.

Historically interest in the gelatinization of the blood dates back as far as 600 B.C. when the separation of shed blood into its component serum, red blood cells, and buffy coat of leukocytes was described.[1] Hippocrates and Aristotle both endeavored to explain the process as solidification associated with cooling. Others of conflicting opinion saw it as a manifestation of response to cessation of the flowing motion of the blood in the circulatory system even though the flow of blood through the vessels was not fully elucidated until the studies of William Harvey many centuries later.

It remained for William Hewson of the school of anatomy of William Hunter to begin to explain the process with understandable

basic principles in the late 1700's. He established stagnation as an important factor in the process and attempted to dispel the notion that agitation delayed coagulation. He even distinguished between intra and extra vascular clotting, established that coagulative properties resided within the plasma rather than the red cell and stressed the important role of the blood vessels in maintaining the blood in a fluid state.

The editor of the London Medical Gazette in commenting upon an article submitted by Andrew Buchanan in the late 1845's suggested the catalytic action of a substance from washed clot which was active in the conversion of fibrin dissolved in solution. However, Virchow in 1856 showed that fibrin could not exist in fluid in an isomeric liquid state. Postulating a precursor state with entirely different characteristics, he called this substance fibrinogen, now Factor I of the coagulation scheme. Thus, the way was set for new discoveries with the hypothesis that the actions of the coagulative process are carried on chemically and the elements eventually involved exist in a precursor state in the blood.

In 1860 Alexander Schmidt named the enzymatic agent from washed clot thrombin and expressed a belief that it was derived from an inactive precursor substance called prothrombin. He further felt that these precursors resided in red cells and other protoplasm calling them zymoplastic agents, which were later to be known as thromboplastins. By 1875 Hammerstein noted the

striking effects of calcium chloride in speeding coagulation. Arthus and Pages at the Sorbonne proved conclusively that calcium salts were essential and in 1900 published a report that the oxalates of sodium, potassium, or ammonium could completely prevent clotting of whole blood. The subsequent addition of calcium brought about clotting of this oxalated plasma.

In 1905 the Classical Theory of Morawitz made its appearance in the German literature. It postulated that fibrinogen, calcium salts, and probably prothrombin were present in the plasma of circulating blood; the presence of anticoagulants (antithrombins); the presence of the enzyme thrombokinase (thromboplastin) in the formed elements, platelets, and leukocytes, which in the presence of calcium could convert prothrombin to thrombin.

Some time later heparin was discovered inadvertently in an attempt to isolate from tissues a cephalin believed to act as an active component in the neutralization of antiprothrombins.

Warner, Brinkhous, and Smith in Iowa in the period from 1930-1940 established that thrombin was the enzyme which converted fibrinogen to fibrin and aided in the establishment of the Morawitz hypothesis by concluding that fibrinogen was not converted to fibrin by washed tissues in one step.

Remaining clotting factors were discovered relatively recently. Dr. Owren in Norway during the Nazi occupation in 1943 studied a young woman with severe hemorrhagic disease. Her clotting defect was not corrected by purified prothrombin and

levels of the known factors I through IV were adequate. He, therefore, postulated the presence of a new factor which he called Factor V. He was able to supply this factor and correct the defect with normal plasma that had been adsorbed with barium sulfate.

Concurrently Dr. Quick in the U.S.A. had noted that prothrombin times by the one stage method performed serially were lengthened over a period of hours and could be corrected by the addition of prothrombin-free fresh plasma. He thus reasoned the presence of a factor lacking in stored plasma and referred to it as labile factor. Fantl and Nance in Australia showed that a purified preparation of prothrombin was converted only very slowly to thrombin by brain extracts and calcium and that the rate could be accelerated by the addition of prothrombin-free treated plasma. They referred to this active factor as prothrombin accelerator. Seegers, who later purified this factor, referred to it as accelerator globulin (Ac globulin).

Thus, three researchers came upon the same factor by divergent means relatively simultaneously pointing up one of the reasons the nomenclature and standardization in this field have been confused and the difficulty encountered in any attempt to fully characterize the clotting factors. Along these lines investigators in 1947 designated as Factor VI a hypothetical substance formed during clotting by the action of thrombin. Later the presence of this substance could not be substantiated and accelerin was dropped

from the official nomenclature.

As a result of the 1920 studies of sweet clover disease in cattle by Karl Paul Link and associates, anticoagulant therapy became a reality. Owen and Bollman in conducting a study of dicoumarolized dogs in 1948 noted a discrepancy between one and two stage prothrombin time tests which could be corrected by the addition of one part in ten of normal plasma. Normal serum free of prothrombin, thrombin, and fibrinogen was also corrective, thus ruling out the possibility of the deficiency of Factor V. (This factor is fully expended in normal clotting and hence is not present in serum.) Later Mann and Hurn found that the level of this substance fell in states of vitamin K deficiency along with prothrombin. It also proved heat and age stable, leading to their designation of stable factor. It remained then for Koller, Loeliger, and Duckert in 1951 to completely separate the factor from prothrombin and show that during therapy with dicoumarol that Factor VII falls faster than that of prothrombin. Using similar techniques Owren arrived at the same conclusions designating his substance as proconvertin. Another series of investigations in 1951 by Alexander and co-workers on a patient with a bleeding disorder supplied the first documented case report. In this case the factor was referred to as serum prothrombin conversion accelerator (SPCA), again pointing up the variety of approaches utilized in the pioneer development of the field of coagulation.

Hemophilia is perhaps the best known of all coagulation

disorders and was responsible for the discovery and characterization of Factor VIII. Patek and Stetson first recognized the true nature of the defect in 1936 by correcting the clotting disorder with a small amount of globulin material obtained by dilution and acidification of plasma.

In 1944 it was demonstrated that the mixing of bloods of certain hemophilic patients brought about mutual correction of their clotting defects. Aggeler and his associates applied the correct interpretation to these findings in 1952 in study of a patient with features indistinguishable from classic hemophilia. They found themselves unable to correct his clotting defects with potent preparations of AHG. After establishing normal levels of other clotting factors previously described, they noted that the abnormality could be corrected by normal serum. Prior treatment with barium sulfate resulted in failure of correction by this serum and the factor involved was called plasma thromboplastin component or Factor IX.

Later a group of scientists demonstrated the deficiency to be inherited in like manner to that of classic hemophilia; thus, establishing the Biggs, Douglas, Macfarlane, Dacie, Pitney, Merskey, and O'Brien syndrome (hypocoprothromboplastinogenemia). Christmas Disease seemed a "legitimate, unassuming, and pleasantly provocative term" for this disorder according to the quotation from Biggs, Douglas, and Macfarlane in defense of the final designation of this disease after the family name of the first

patient studied.

The remaining three factors were then discovered rather rapidly. The Stuart-Prower Factor was discovered in 1956 and has strong resemblance to Factor VII with the exception that its deficiency brings prolongation of the whole blood clotting time and is corrected by the venom of the Russell viper from Southeast Asia.

The term plasma thromboplastin antecedent (PTA) was coined in 1953 by Rosenthal. This factor acts early in coagulation in the process of surface activation and was discovered in examination of a hemorrhagic disorder in a patient.

A prolonged clotting time determination on a patient named Hageman led to the discovery of Factor XII by Ratnoff in 1955. The patient had no hemorrhagic symptoms and the Hageman Factor is believed similar to Factor XI and involved in the surface activation system. It is difficult to separate the two factors, and it is thought they may interact to form an intermediate product active in formation of thromboplastin.

Having now provided some insight into the circumstances surrounding the discovery of the factors concerned, it is necessary to provide a schema whereby they become involved in a mechanism explaining their integrated action. Using an elaboration of the process first outlined by Morawitz, expanded to include the more recent facts, a reasonable outline of the process can be presented.[2] A chart listing various alternate names of

each factor is provided (see Appendix I).

Stage I - Thromboplastin Generation

A. Extrinsic or tissue thromboplastin

Tissue Extracts

Factor VII — Calcium ⟶ Active tissue thromboplastin

Factor X

Factor V

This is a rapid process thought to require only 6-8 seconds. It is felt that small amounts of thrombin formed by the tissue thromboplastin serve as accelerators for the blood thromboplastin.

B. Intrinsic or Blood Thromboplastin

Preliminary Step:

Factor XI
Factor XII — Contact / Calcium ⟶ Active PTA(XI)

This phase has been referred to as the lag phase (Duckert, 1960) and normally requires from 5-9 minutes *in vitro*.

Step 1

Factor IX
Factor VIII
Active PTA (XI) — X (Stuart) / 2-4 minutes ⟶ Intermediate product I

Step 2

Intermediate product I
Platelet Factor III — seconds ⟶ Intermediate product II

Step 3

Intermediate product II
Factor V $\xrightarrow[\text{seconds}]{\text{?Calcium}}$ Blood thromboplastin

Stage II - Thrombin Generation

Prothrombin
Thromboplastin $\xrightarrow[\text{4-8 seconds}]{\text{V, X, Calcium}}$ Thrombin

Stage III - Fibrin Formation

Fibrinogen
Thrombin $\xrightarrow{\text{4-8 seconds}}$ Fibrin

Further expansion of the role of these factors will be accomplished later in the discussion of each individually.

With this information at hand, one may now set out to analyze the findings of the various investigators of the subject of coagulation during pregnancy. The recent discovery and difficulties in separation and quantitation of some of these factors has led to an obvious gap in knowledge concerning their relation to pregnancy. Few authors have attempted any explanation as to the reasons why these changes occur and much remains undone in correlating the complex and interwoven chemical and physiologic status in pregnancy with the changes in the formed elements and molecular structures of the clotting system. For the time many questions regarding the precise character of the coagulation proteins, their relation to placental transport, antibody formation, etc., must go unanswered.

One can, however, by confining himself to the better established facts, analyze the status of the twelve factors of the coagulation system during pregnancy. Attempt is made to refer as often as possible to these factors using the Roman numeral assigned it; but realizing the multiplicity of names attached to certain factors, a chart is provided in the appendix listing synonymous names which occur in the literature.

Factor I (fibrinogen) is a plasma protein euglobulin; the concentration of which varies somewhat depending upon the method of quantitation used. Ratnoff offers a mean value of 294 mg. per 100 ml., and most others are in agreement that the level is approximately 300 mg. per 100 ml. in the nonpregnant state.[3] During pregnancy the level rises to a mean of 370 mg. per 100 ml. at 13-16 weeks and eventually to an average of 440 mg. per 100 ml. at 37 weeks gestation and thereafter.[4] Values as high as 600 mg. per 100 ml. are not unusual.[5] A case of hyperemesis gravidarum with development of a severe bleeding disorder at the third month of gestation was reported with a fibrinogen level of 856 mg. per 100 ml.

The mechanism of the rise is not clear, and Dr. Rosenblum at the Western Reserve Medical School has attempted to link the rise to known endocrine changes but has enjoyed no clear-cut success thus far. The rise appears responsible for the increased erythrocyte sedimentation rate in pregnancy, and concentrations of fibrinogen are known to be increased by pituitary growth hormone

and decreased by cortisone. A round table conference on hemostasis in Amsterdam in 1962 reported that estrogens were able to increase fibrinogen levels on a therapeutic basis.[7]

Factor II (prothrombin) is certainly one of the most widely studied of the coagulation factors through its connection with vitamin K and hepatic function. It is a protein which migrates in an electrophoretic field as an alpha-2 globulin and has a molecular weight of 63,000. It is produced in the liver and is vitamin K dependent.

There is incomplete agreement regarding the activity of this factor during pregnancy; some investigators report no change whatsoever.[8] The concensus, however, reveals a small but consistent increase in prothrombin level and activity through the course of pregnancy. In a Mayo Clinic Study the prothrombin time dropped from 18 seconds (normal 17-19) at onset of pregnancy to 16 seconds at term.[8] Others were able to substantiate these findings with Talbert and Langdell reporting figures of 13.5 seconds to 11.2 seconds from 60 days to term respectively.[5]

Prothrombin levels are variously reported as 111% of normal nonpregnant values (12 mg%) in one study[9] and 120% in others.[3,4] Lowest levels were found immediately post partum and during the six hours thereafter. Values had all returned to ante partum levels between 3 and 7 days post partum and had returned to normal by 6 weeks.[9]

Estrogens are reported to increase prothrombin activity.[7]

Factor III (thromboplastin) includes a group of substances which exhibit thromboplastic activity and are thought to be predominantly lipoproteins of rather large molecular weight containing cephalin or other phospholipids. Different body tissues vary considerably in thromboplastic activity with brain, lung, placenta, decidua, amniotic fluid, and blood platelets among the richest sources.

Alterations in this system are difficult to assess and interpretations must be based on the results of complex interactions. Units have been devised and can be used for assay procedures. Platelet levels are open to some question in regards to their behavior during pregnancy, but the count is probably not significantly altered.[2]

Phospholipids are known to increase during pregnancy and coupled with the shortened coagulation times in pregnancy would suggest an accelerated activity.[11] No specific information was found, however.

Factor IV (calcium) in its ionic form is necessary in the formation of thromboplastin and of thrombin in the first and second stages of coagulation, but there are no proven cases of in vivo alterations in the coagulation process as a result of altered calcium levels.

Factor V (labile factor) is a globulin formed by the liver[12] and is dependent upon vitamin K. Activity is rapidly lost at room temperature, particularily in the presence of oxalates. It

functions as an accelerator to stages one and two of coagulation, being entirely expended in clotting and, therefore, not found in serum. It is not adsorbed by barium sulfate.

There is general agreement regarding the lack of significant alteration of this factor during the course of pregnancy.[5,8,13] It is, however, one of the few factors elevated in the newborn.

One group was found which reported increased values (mean of 180% of nonpregnant values) in their series of 41 normal and 90 pregnant women.[9] The Amsterdam Conference on hemostasis in 1962 also reported that Factor V levels were "significantly" elevated as a result of estrogen administration; however, this is certainly widely disputed, and the work with estrogen therapy is not as yet completely consistent.[7]

A series of very interesting studies by Egeberg in Oslo on the response of the coagulation mechanism to physiologic alterations produced an apparent elevation of Factor V during diuresis and during the mobilization of edema fluid.[14] These two phenomena are not unfamiliar in the late gestation period.

Factor VII (stable factor) is a beta globulin and can be found in both plasma and serum. It functions as an accelerator to the conversion of prothrombin to thrombin in combination with Factor V, tissue extracts, and calcium ions. It is vitamin K dependent and, therefore, thought to be formed in the liver. It is depressed by dicoumarol therapy and responds much more rapidly than prothrombin both in depression on initiation of therapy and

in return to normal levels upon its discontinuation. Half-life is estimated at about 5 hours, slightly longer than the labile Factor V.[15] It is adsorbed by barium sulfate, and in vitro modification of defects can be accomplished with Russell's viper venom.

In pregnancy the levels of Factor VII are significantly increased in the findings of nearly all investigators. This change reaches maximum levels during the last trimester with mean values in the Mayo Study varying between 254 and 625% of normal values (S.D. 17%).[8] The data of other authors is consistent with these findings.[3,4,10,13,16,17]

The specific effect of estrogen on Factor VII levels is less clear-cut, and no consistent trend could be interpreted.[7,19,20,21]

Factor VIII (AHG) is a protein beta globulin noted for its deficiency in classic hemophilia of type A. It can be found only in plasma and functions in thromboplastin generation within the intrinsic system of the first stage of clotting. In vivo half-life is estimated at 6-12 hours.[15] It is, therefore, quite labile and does not persist in bank blood on storage with loss of 40 to 70% of activity during the average three week storage period. Barium sulfate will not adsorb Factor VIII.

Wide variation can be found in the opinion regarding behavior of Factor VIII during pregnancy with many reporting no change in activity. There are impressive reports, however, which detail increased levels, such as that by H. S. Strauss and Diamond

reporting a series of normal patients and one with Von Willebrand's Disease.[6] These investigators reported that, contrary to common opinion, there was a rise in Factor VIII in both normal women and their deficient patient; though admittedly the rise demonstrated much individual variability. These patients were studied for a 6-8 week period during pregnancy, on the day of parturition, and with cord blood samples using a modification of the thromboplastin generation test. The Mayo Study of M. E. Todd also demonstrated elevation of Factor VIII levels using a retarded thromboplastin generation test which used aluminum hydroxide to distinguish thromboplastin generation accelerators from Factor VIII. They also employed direct assay methods and, although the two methods failed to correlate ideally, they demonstrated levels as high as 450% of normal.[8] In yet another study levels of Factor VIII in normal and AHG deficient women were again determined and again elevated levels were discovered in both.[13]

The influence of metabolic activity on Factor VIII was demonstrated by Egeberg with "abnormally high" values in hyperthyroid patients.[22] Exercise (20 stairs for 20 minutes) also produced increased values as did diuresis and the mobilization of edema fluid.[14,23] All of these conditions are either seen or mimiced in normal pregnancy.

Factor VIII also appears to cross the placental barrier, sharing this distinction only with platelet agglutinins among coagulation products.[24]

Factor IX (PTC) is a beta globulin of the intrinsic system active in the formation of thromboplastin. It is absent in hemophilia B and is transmitted as a sex-linked recessive gene as is Factor VIII. It is heat labile with a half-life estimated at 20-30 hours,[15] appears to be synthesized by the liver, is vitamin K dependent, and is depressed by dicoumarol therapy. It is adsorbed by barium sulfate.

Factor IX activity has its effect upon the amount of thromboplastin produced but does not appear to influence the rate of formation.

During pregnancy the level of Factor IX appears to be increased in the range of 10%.[2,3,8,13] This factor has not been extensively investigated in pregnancy.

Factor X (Stuart-Prower) is an alpha globulin which is required for generation and functioning of thromboplastin in both intrinsic and extrinsic systems. It appears to catalyze the reaction between Factors VIII, IX, and "active" PTA (XI).

It is vitamin K dependent and, therefore, thought to be manufactured in the liver and is depressed by dicoumarol therapy. Half-life is judged to be 2-3 days[15] and it is adsorbed by barium sulfate. Factor X is required for the satisfactory corrective action of Russell's viper venom.

This factor influences the rate of thromboplastin formation, but not the amount formed.

In pregnancy the level rises to the range of 145-400% of

normal according to the Mayo Study.[8] An interesting report by S. Haber in the Archives of Internal Medicine describes a case of Factor X deficiency responsive clinically during repeated pregnancies and later to therapy with norethynodrel (progesterone agent) during an exacerbation of hemorrhage.[21] Several other studies were in agreement regarding the elevated levels[13,16] with no real disagreement among the papers reviewed. A 30% increase was measured in a quantitative study by Pechet and Alexander in 1961.[11]

Factor XI (PTA) is a beta globulin which functions in the intrinsic system of the first stage of coagulation and is essential to thromboplastin generation. It functions in the surface activation mechanism in combination with Factor XII, and it is difficult to separate from it. In contrast to Factor XII it has been known to be responsible for bleeding when deficient. This factor is found in serum and is thought to be stable in stored bank blood. Barium sulfate adsorption is incomplete. A condition referred to as Hemophilia C Disease has Factor XI as a primary deficit.

No specific information could be found in the literature regarding levels and activity with pregnancy. In most cases it was measured in combination with Factor XII, which does show an increase in activity.

In the experiments of Egeberg with edema and diuresis Factor XI appeared to decrease despite the increased levels of

Factors VIII, V, and I.[14]

Factor XII (Hageman) is a protein which migrates between the beta-2 and gamma globulins in electrophoretic fields. It probably plays no part in coagulation defects in vivo but functions in the intrinsic system of thromboplastin formation being activated on surface contact and is similar in action to Factor XI from which it is separated with difficulty. It appears stable in stored bank blood and is partially adsorbed by barium sulfate.

There is evidence of increased activity with pregnancy,[3] but this is not well documented nor does it appear to be of particular consequence at this time.

To summarize the previous data it would seem that the coagulation factors behave in a characteristic manner during the course of normal pregnancy for the most part, the major exception being Factor VIII, which at this point has been somewhat inconsistent. Certain of them are increased significantly, those being Factors I, VII, VIII, and X. Others show consistent increase but on a lesser scale of magnitude, namely Factors II, III, and IX. Factor V holds a position as the one factor showing no significant alteration in activity or concentration. So far no significant evidence has been accumulated regarding the activity of Factors XI and XII. No factor has demonstrated a tendency toward decrease with normal uncomplicated pregnancy.

In conjunction with the aims stated previously a series of pregnant women were selected at random from the patients of the

Obstetrics Clinic at the University of Nebraska Hospital. None of these women had prior history suggestive of bleeding disorder. Ages ranged from 17 to 35 years and no regard was given to race. Approximately one-third were pregnant for the first time, and two-thirds had one or more and as many as 9 living children. Attempt was made to eliminate those uncertain regarding time of conception so that only women within a predicted two-week period from delivery were finally included.

The laboratory determinations selected were three: partial thromboplastin time, prothrombin time, and plasma fibrinogen level. Determinations were according to the routine protocol of the Department of Pathology, University of Nebraska Hospital, and the procedures used for the partial thromboplastin time and plasma fibrinogen determinations are appended. (See Appendices II and III.) Prothrombin times are performed by the one stage method of Quick using a commercially prepared source of thromboplastin.

These tests were chosen for several reasons; paramount among them is the fact that each is a particularly good test for the stage of coagulation for which it is concerned: the partial thromboplastin test for Stage I, prothrombin time in Stage II, and fibrinogen level in Stage III. These tests offer simplicity of determination and are adaptable to ready determination by laboratory personnel of fairly minimal special training as compared to many coagulation tests. This factor makes them available on a round-the-clock schedule should need arise, and they are relatively

inexpensive to perform. Another advantage is that they cover a broad spectrum of situations in medical practice; the partial thromboplastin time has demonstrated itself a very satisfactory test for all the common deficiency states excluding only Factor VII, which rarely presents as a single deficiency.

The Quick prothrombin time is capable of detection of deficiency of Factor VII as well as Factors II, V, and X, using a "complete" thromboplastin.

Fibrinogen is the factor most frequently found deficient in those complications of pregnancy leading to bleeding diathesis. These include the premature placental separation of either placenta previa or classic abruptio placenta. Prolonged retention of a dead fetus (3-4 weeks), pre-eclampsia, eclampsia, criminal abortion, and amniotic fluid embolism are also included in this group. Nearly every other obstetric disorder associated with shock has also been associated with hypofibrinogenemia. Relatively recent information regarding these conditions as related to coagulation disorders can be gained by reading Ratnoff and Holland from Western Reserve University[4] or the chapter dealing with hemorrhagic disorders of pregnancy in the 1963 book of Dr. Cecil Hougie from the University of Washington.[3]

The results of the laboratory determinations are compiled in Appendix IV and will be summarized. Twenty patients were submitted to plasma fibrinogen determination with a maximum level of 585 mg. per 100 ml. and a minimum figure of 260 mg. per 100 ml. The mean

value was 395 mg. per 100 ml. which compares favorably with those presented in the literature. The Mayo Study reported a mean of 419 mg. per 100 ml. during the ninth month. There is wide latitude in the range of this determination as evidenced by the standard deviation of 78 mg. per 100 ml. and the standard laboratory normal range of 200-400 mg. per 100 ml. In this series no deficiencies were detected.

Partial thromboplastin time was determined on the plasma of 19 women. These levels show a consistent pattern with a range of 75-44 seconds. The mean for the entire group is 56 seconds with a standard deviation of 7.2 seconds. This compares with an average of 84.4 seconds in a study of 1,096 normal individuals by Dr. John Miale.[25] Using a similar technique, standard deviation in his study is 10.1 seconds. Another series of 206 normal persons revealed an average value of 76 seconds with a standard deviation of 13 seconds.[26] Shortest times quoted revealed a value of 67 seconds with a standard deviation of 6 seconds using their laboratory preparation of partial thromboplastin on 10 normal subjects.[26]

Thus, with the rather fixed reaction times of the latter two stages of the coagulation mechanism, this obvious acceleration must be predominantly in the first stage in response to the increased levels of substrates and accelerators found in late pregnancy. Specific actions of those factors were detailed earlier.

Control values listed for this procedure are the standardized normal plasma (SNP) values of the Dade reagent freshly reconstituted for each set of determinations.

Prothrombin time values revealed no disorders of the factors related to this test. One patient did show what is commonly accepted as a significant elevation of prothrombin time with a 15 second reading which was 3 seconds longer than the control. This patient as yet has shown no clinical signs of deficiency or disease and has no compatible history but will be studied further. The overall values varied between 11 and 15 seconds with an average of 11.9 seconds as compared to a control value of 11.4 seconds. It is felt that these figures present no unusual findings in that with the absence of deficiency of the necessary factors the reaction time is rather fixed.

In conclusion then, it would seem that the coagulation system during pregnancy is truly rendered "hypercoagulable" in that the majority of the factors concerned are increased in concentration or activity. It has been suggested that the major increase in Factors VII and fibrinogen during pregnancy are "probably meaningless" with respect to starting spontaneous intravascular clotting but can serve a useful purpose when tissue thromboplastin becomes available as from the placenta.[17] There is, however, in partial refutation of this suggestion the evidence that during a great many circumstances of stress (for example, surgical operations) circulating ionic fatty acids and

total lipids are increased with concomitant shortening of the clotting time.[27] Thus possibly the stage is set for a hyperirritable physiologic response which with the proper stimulation can become pathologic. Thus is the case of highly undesirable thromboses frequently encountered at some stage of pregnancy, delivery, or puerperium. These facts are sufficient to warrant considerably greater interest in the colloid-osmotic status of the obstetric patient at the various intervals of her pregnancy and parturition and perhaps suggests even more specific measures. These would include those normally followed in minimizing stasis and anoxia and so forth, but might also prove a very credible rationale for providing the fat and protein sparing effects of carbohydrate administration rather routinely during periods of stress. Labor and delivery represent such stress, even when uncomplicated.

With the evidence stated in the foregoing material perhaps some mention of potential etiology for these changes might be made. There are many physiologic alterations in pregnancy that now seem quite distinctly related to the coagulation process. In the category of hormone alterations the estrogens have been used extensively as a therapeutic measure to combat bleeding diatheses. A case report was earlier quoted in which a progesterone agent (norethynodrel) was used to treat a deficiency state of Factor X after the condition was noted to improve during repeated pregnancies of the patient.[21] Certainly then there is strong

suggestion that the hormonal elevations in pregnancy have a part in the changes in the coagulation mechanism.

The work of Egeberg adds evidence in this category with the finding that increased thyroid function could be correlated with increased activity of the intrinsic plasma coagulation system.[22] He went further to attribute these changes to the increased tissue metabolic state and in studying exercise found this had a similar though less remarkable result.[23] Potential avenues of explanation here are too numerous to mention.

In the category of body fluid alterations there is evidence that venous congestion and resultant stasis enhance coagulation activity through effects including concentration of proteins and loss of fluid from tissues. This is coupled with release of a thromboplastinlike material from these tissues.[28] Also mentioned was the stimulatory effect of edema and the mobilization of edema fluid on the coagulation accelerators.[14]

Other factors worthy of consideration in the creation of a "hypercoagulable" state include proteins and lipids. The majority of the coagulation substances are in fact proteins and, in addition, there is the theory that a high protein diet has been correlated with a decrease in the clotting time.[29] Lipids are involved through the lipoprotein character of the thromboplastic substances. Certainly there is debate regarding the true nature of the coagulation changes with lipemic states, but many have felt a correlation with increased <u>in vitro</u> coagulability could be made.

Related to this problem is the relation of stress which is likewise unresolved; however, it is safe to state that the pregnant state is associated with periods of active stress.

In summary the attempt has been made to broadly cover the subject of coagulation in pregnancy. The problem was explored to evaluate its significance, the recent literature was reviewed and summarized, and a series of term pregnant women were examined for overall coagulation status. Although the individual levels show considerable variation, there are consistent overall trends. Interpretation of these trends stands on somewhat tremulous grounds; however, much evidence is accumulating and demands interpretation in the formulation of a working impression. Therefore, an attempt was made to present the evidence as discovered with the interpretation held by this author with sufficient reference to allow the critic to form a separate opinion. This is not only his right but his duty in evaluating medical literature.

APPENDIX I

Alternate Terms for Coagulation Factors

Factor I	Fibrinogen
Factor II	Prothrombin
Factor III	Thromboplastin
	Tissue thromboplastin
	Thromboplastic substance
	Kinase
	Thrombokinase
	Cytozyme
	Zymoplastic substance
Factor IV	Calcium
Factor V	Labile factor
	Ac globulin (Ac glob)
	Proaccelerin
	Prothrombin accelerator factor
	Plasma prothrombin converting factor
Factor VI	Dropped (Accelerin)

APPENDIX I (continued)

Factor VII	Stable factor
	Serum prothrombin conversion accelerator (SPCA)
	Convertin
	Proconvertin-convertin
	Prothrombinogen
	Prothrombokinase
	Cothromboplastin
	Prothrombin conversion accelerator (PCA)
	Serum Ac globulin
	Serum accelerator
Factor VIII	Antihemophilic factor (AHF)
	Antihemophilic globulin (AHG)
	Platelet cofactor I
	Hemophilic factor A
	Thromboplastinogen
	Thrombocytolysin
Factor IX	Plasma thromboplastin component (PTC)
	Christmas factor
	Hemophilic factor B
	Antiprothrombin II
	Platelet cofactor II
	Thromboplastinogen

APPENDIX I (continued)

Factor X	Stuart Prower factor
	Stuart factor
Factor XI	Plasma thromboplastin antecedent (PTA)
	Hemophilic factor C
	Cothromboplastin
Factor XII	Hageman factor
Fibrinolysin	Plasmin
Profibrinolysin	Plasminogen

APPENDIX II

The Partial Thromboplastin Time Test (Cephalin Time)

Macro Test

Reagents:

1. Dade Cephaloplastin.
2. Calcium chloride, 0.02M: Dissolve 0.222 gm. of anhydrous calcium chloride in distilled water and dilute to 100 ml.
3. Sodium oxalate, 0.1M: Dissolve 1.34 gm. anhydrous sodium oxalate in distilled water and dilute to 100 ml.

   OR

   Sodium citrate 0.1M: Dissolve 2.94 gm. of sodium citrate ($NA_3O_6H_5O_7 \cdot 2H_2O$) in distilled water and dilute to 100 ml.
4. Normal control: Dade SNP (Standardized Normal Plasma) freshly reconstituted.

Procedure:

1. Combine 9 parts of freshly collected blood with 1 part of 0.1M sodium oxalate, or 0.1M sodium citrate.
2. Mix well and centrifuge for 5 minutes at 2,000 rpm. Remove supernatant plasma and store in refrigerator until ready to use. Plasma should be tested within 2 hours after collection.
3. Place tube of 0.02M calcium chloride in 37°C. waterbath.
4. Pipet 0.1 ml. of Cephaloplastin into desired number of tubes and place in waterbath. Allow to incubate for 1-2 minutes.
5. Add 0.1 ml. of plasma (SNP or patient's) to cephaloplastin. Mix well and allow to incubate for 1 minute at 37°C.
6. Forcibly blow 0.1 ml. of 0.02M calcium chloride into the cephaloplastin-plasma mixture; simultaneously start stopwatch.
7. Incubate tube in waterbath for 30 seconds, remove tube and observe for clot formation.

NOTE: The wide variation in the normal time makes it difficult to estimate exactly when to remove the test from the waterbath, It is suggested that the first determination be used as an approximate time. Allow repeated determinations to incubate to within 20 seconds of the expected time before removing from the waterbath.

Normal is 40-80 seconds.

If partial thromboplastin time is between 80-100 seconds, the test should be repeated with freshly collected plasma.

Patients giving prolonged times may require more specific tests such as the Quick Prothrombin Consumption Test with its modifications,[9] or the TGT, in order to identify the deficient factor.

APPENDIX III

Plasma Fibrinogen Determination

References: Technical Bulletin, 25, p. 48, 1955.
Arch. Biochemistry, 46, p. 470-480, 1953.

Principle: Fibrinogen is a globulin more easily precipitated than the other plasma globulins. It is practically quantitatively precipitated by 12% $(NH_4)_2SO_4$. The plasma is mixed with an appropriate salt solution (Parfentjev's reagent) and the resulting turbidity is read in a photometer against the same plasma diluted in 0.9% saline. The fibrinogen is calculated by means of an equation determined empirically using purified fibrinogen diluted with fibrinogen free serum.

Reagents:

1. 0.9% NaCl.
2. Parfentjev's reagent - In a liter volumetric flask place 133.33 gm. $(NH_4)_2SO_4$, 10.0 gm. NaCl, 0.025 gm. merthiolate, and dilute to volume. Adjust pH to 7.0 with 10 M NaOH.

Procedure:

1. Collect 5.0 ml. of blood in a tube containing 0.5 ml. of 4% sodium citrate $(2H_2O)$.
2. Centrifuge at 2500 rpm for 10 minutes in order to obtain supernatant plasma.
3. Prepare 2 tubes as follows:

Patient	Blank
0.5 ml. plasma	0.5 ml. plasma
4.5 ml. Parfentjev reagent	4.5 ml. 0.9% saline

4. Allow to stand exactly three minutes, shake before pouring into small cuvette and read optical density with blank set at 0 at 510mu.
5. The fibrinogen concentration of the sample is given in grams% by the equation:

$$\text{gms.\% fibrinogen} = \frac{\text{O.D.} + 0.019}{0.509}$$

1000 x gm% = mgm% fibrinogen

NORMAL = 200-400 mgm%.

Note: Use small cuvettes.

APPENDIX IV

Summary of Coagulation Studies

Patient Number	Fibrinogen mg%	Macro PTT sec.	Control PTT sec.	Prothrombin sec.	Control sec.
1	336	54	70	11	11
2	585				
3	354	58	56	12	11
4	357	44	63	13	12
5	373	53	64	12	12
6	375	60	70	12	11
7	300	54	56	11	11
8	449	56	70	11	11
9	465	56	70	11	11
10	462	63	74	11	11
11	387	66	56	11	11
12	465	52	71	11	11
13	373	54	74	11	11
14	260	57	74	12	11
15	398	57	62	13	12
16	312	75	58	12	12
17	366	49	62	15	12
18	373	44	39	12	12
19	528	56	64	12	12
20	379	51	64	13	12
Mean	395	56	64	11.9	11.4
S.D.	78	7.2	8.7	1.004	1.6

BIBLIOGRAPHY

1. Hougie, Cecil, Fundamentals of Blood Coagulation in Clinical Medicine, New York, Blakiston Company, 1963, Chapter 1.

2. Phillips, L. L., Modifications of the Coagulation Mechanism during Pregnancy, Modern Trends in Human Reproductive Physiology, 1:190-204, 1963.

3. Hougie, Cecil, Fundamentals of Blood Coagulation in Clinical Medicine, New York, Blakiston Company, 1963, Chapter 13.

4. Ratnoff, O. D. and Holland, R., Coagulation Components in Normal and Abnormal Pregnancies, Ann. N.Y. Acad. Sci., 75:626, 1959.

5. Talbert, L. M. and Langdell, R. D., Normal Values of Certain Factors in the Blood Clotting Mechanism in Pregnancy, Amer. J. Obstet. Gynec., 90:44-50 (Sept.) 1964.

6. Bailey, P., Vitamin K Deficiency in Early Pregnancy, Brit. Med. J. 5418:1199 (Nov.) 1964.

7. Excerpta Medica Foundation, Hemostasis and the Effect of Estrogens: Transcript of a round table conference, Amsterdam, 1962.

8. Todd, M. E. and others, Blood Coagulation During Pregnancy, Mayo Clin. Proc. 40:370-383 (May) 1965.

9. Alexander, B. and others, Blood Coagulation in Pregnancy: Proconvertin and Prothrombin and the Hyper Coagulable State, New Eng. J. Med., 254:358, 1956.

10. Schwenzer, A. W. and others, Das Gerinnungs-system unter der Geburt, Arch. Gynäk, 191:8, 1958.

11. Pechet, L. and Alexander, B., Increased Clotting Factors in Pregnancy, New Eng. J. Med., 265:1093, 1961.

12. McKay, D. G., Disseminated Intravascular Coagulation: An Intermediary Mechanism of Disease, New York, Hoeber Med. Div., Harper and Row, 1965, Chapter 2.

13. Kasper, C. K. and others, Blood Clotting Factors in Pregnancy: Factor VIII Concentrations in Normal and AHF Deficient Women, Obstet. Gyned. 24:242-247 (August) 1964.

BIBLIOGRAPHY (continued)

14. Egeberg, O., Effect of Edema Drainage on the Blood Clotting System, Scandinav. J. Clin. and Lab Investigation, 15, 13-20, 1963.

15. Salzman, E. W., Hemorrhage and Thrombosis, Boston, Little Brown and Company, First Edition, 1965, p. 9-12.

16. Strauss, H. S. and Diamond, L. K., Elevation of Factor VIII (Antihemophilic Factor) During Pregnancy in Normal Persons and in a Patient with Von Willebrand's Disease, New Eng. J. Med. 269:1251-2 (Dec.) 1963.

17. Mammen, E. F., Physiology of Blood Coagulation in Pregnancy, Clin. Obst. and Gynec., 7, 271 (June) 1964.

18. Beuer, F. K., Die Gerinnungsverhältnisse bei der Schwangerenund beim Neugeborenen, Barth, Leipzig, 1957.

19. Johnson, J. F., Changes in Plasma Prothrombin, Ac Globulin, and Antithrombin Concentration Following Intraveneous Administration of Estrogens, Proc. Soc. Exp. Biol. Med. 94:92, 1957.

20. McGovern, J. J. and others, Effects of Conjugated Estrogens on Coagulation Mechanism, JAMA, 175:1011, 1961.

21. Haber, S., Norethynodrel in the Treatment of Factor X Deficiency, Arch. Intern. Med. 114:89-93 (July) 1964.

22. Egebert, O., Influence of Thyroid Function on the Blood Clotting System, Scandinav. J. Clin. and Lab. Investigation, 15, 1-7, 1963.

23. _____, The Effect of Exercise on the Blood Clotting System, Scandinav. J. Clin. and Lab. Investigation, 15, 8-13, 1963.

24. Schaffer, A. J. and others, Diseases of the Newborn, 2nd Edition, Philadelphia, W. B. Saunders Company, 1965, Chapter 66.

25. Miale, J. B., Routine Evaluation of Bleeding Tendency in Surgical Patients, JAMA, 185:752-756 (Sept.) 1963.

26. Nye, S. W. and others, The Partial Thromboplastin Time as a Screening Test for the Detection of Latent Bleeders, Am. J. Med. Sci., 243:279-287 (March) 1962.

27. Wainwright, N. D., Stress and Its Effect on the Coagulability of Blood, Senior Thesis (February) 1965.

28. Egeberg, O., Effect of Venous Congestion on the Blood Clotting System, Scandinav. J. Clin. and Lab. Investigation, 15, 21-28, 1963.

29. Dieckmann, W. J., Changes in Principle Blood Constituents During Pregnancy, Second Edition, St. Louis, C. V. Mosby, 1952, p. 98.