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University of Minnesota Hospitals
and
Minnesota Medical Foundation

Blood Clotting
Recent Advances

Volume XX | Friday, May 27, 1949 | Number 29

BULLETIN OF THE
UNIVERSITY OF MINNESOTA HOSPITALS
and
MINNESOTA MEDICAL FOUNDATION

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INDEX

PAGE

I. CALENDAR OF EVENTS . . . . 586 - 588

II. BLOOD CLOTTING -- RECENT ADVANCES . . . . 589 - 611

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III. MEDICAL SCHOOL NEWS 612

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I.

UNIVERSITY OF MINNESOTA MEDICAL SCHOOL
CALENDAR OF EVENTS

May 29 - June 4, 1949

No. 250

Sunday, May 29

9:00 - 10:00 Surgery Grand Rounds; Station 22, U. H.

Monday, May 30 - HOLIDAY

Tuesday, May 31

8:00 - 9:00 Fracture Conference; Auditorium, Ancker Hospital.

8:30 - 10:20 Surgery Seminar; Carcinoma of the Stomach; G. G. Lenz; Small Conference Room, Bldg. I, Veterans Hospital.

9:00 - 9:50 Roentgenology Pediatric Conference; L. G. Rigler, I. McQuarrie and Staff; Todd Amphitheater, U. H.

10:30 - 11:50 Surgical Pathological Conference; Lyle Hay and Robert Hebbel; Veterans Hospital.

12:30 - Pediatric-Surgery Rounds; Sta. I, Minneapolis General Hospital; Drs. Bosma, Wyatt, Chisholm, McNelson, and Dennis.

12:30 - 1:20 Pathology Conference; Autopsies; Pathology Staff; 102 I. A.

1:00 - 2:30 X-ray Surgery Conference; Auditorium, Ancker Hospital.

2:00 - 2:50 Dermatology and Syphilology Conference; H. E. Michelson and Staff; Bldg. III, Veterans Hospital.

3:15 - 4:20 Gynecology Chart Conference; J. L. McKelvey and Staff; Station 54, U. H.

3:30 - 4:20 Clinical Pathological Conference; Staff; Veterans Hospital.

4:00 - 5:00 Pediatric Rounds on Wards; I. McQuarrie and Staff; U. H.

4:00 - 5:30 Physiology-Surgery Conference; Diabetogenic Mechanisms; N. Lifson, S. Barron, and C. Peterson; Eustis Amphitheater, U. H.

5:00 - 5:50 Urology-Pathological Conference; C. D. Creevy and Staff; Todd Amphitheater, U. H.

5:00 - 6:00 X-ray Conference; Presentation of Cases by Dr. Aurelius and Staff, Ancker Hospital; Todd Amphitheater, U. H.

Wednesday, June 1

8:00 - 8:50 Surgery Journal Club; O. H. Wangensteen and Staff; M-515 U. H.

8:30 - 9:30 Clinico-Pathological Conference; Auditorium, Ancker Hospital.

8:30 - 10:00 Orthopedic-Roentgenologic Conference; Edward T. Evans, Room 1AW, Veterans Hospital.

8:30 - 12:00 Neurology Rehabilitation and Case Conference; A. B. Baker and Joe R. Brown; Veterans Hospital.

11:00 - 12:00 Pathology-Medicine-Surgery Conference; O. H. Wangensteen, C. J. Watson and Staff; Todd Amphitheater, U. H.

12:00 - 12:50 Radio-Isotope Seminar; Current Literature; George E. Moore; Rm. 212, Hospital Court, Temp. Bldg.

3:30 - 4:30 Journal Club; Surgery Office, Ancker Hospital.

4:00 - 5:00 Infectious Disease Rounds; E-101, University Hospitals.

Thursday, June 2

8:15 - 9:00 Roentgenology-Surgical-Pathology Conference; Craig Freeman and H. M. Stauffer; M-109, U. H.

8:30 - 10:20 Surgery Grand Rounds; Lyle Hay and Staff; Veterans Hospital.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; M-109, U. H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:50 Surgery-Radiology Conference; Daniel Fink and Lyle Hay; Veterans Hospital.

11:00 - 12:00 Cancer Clinic; K. Stenstrom and A. Kremen; Todd Amphitheater, U. H.

11:30 - 12:30 Clinical Pathology Conference; Steven Barron, C. Dennis, George Fahr, A. V. Stoesser and Staffs; Large Class Room, Minneapolis General Hosp.

12:00 - 1:00 Physiologial Chemistry Seminar; Malonate Inhibition of Oxidations in Krebs Cycle; Oddvar Nygaard; 214 M. H.

1:00 - 1:50 Fracture Conference; A. A. Zierold and Staff; Minneapolis General Hospital.

2:00 - 3:00 Errors Conference; A. A. Zierold, C. Dennis and Staff; Large Class Room, Minneapolis General Hospital.

4:00 - 5:00 Bacteriology and Immunology Seminar; Lactic Acid Fermentation at Controlled Ph Levels; R. K. Finn; 214 M. H.

4:30 - 5:20 Ophthalmology Ward Rounds; Erling W. Hansen and Staff; E-534, U. H.

5:00 - 6:00 X-ray Seminar; Study of Contrast Media; Osmond Baggenstoss; Todd Amphitheater, U. H.

Friday, June 3

8:30 - 10:00 Neurology Grand Rounds; A. B. Baker and Staff; Station 50, U. H.

9:00 - 9:50 Medicine Grand Rounds; C. J. Watson and Staff; Todd Amphitheater, U.H.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:30 - 11:20 Medicine Grand Rounds; Veterans Hospital.

10:30 - 11:50 Otolaryngology Case Studies; L. R. Boies and Staff; Out-Patient Department, U. H.

11:00 - 12:00 Surgery-Pediatric Conference; C. Dennis, O. S. Wyatt, A. V. Stoesser and Staffs; Minneapolis General Hospital.

11:30 - 12:50 University of Minnesota Hospitals General Staff Meeting; Biochemical Effects of Radiation; Samuel Schwartz; Powell Hall Amphitheater.

12:00 - 1:00 Surgery Clinical Pathological Conference; Clarence Dennis and Staff; Large Classroom, Minneapolis General Hospital.

1:00 - 1:50 Dermatology and Syphilology; Presentation of Selected Cases of the Week, H. E. Michelson and Staff; W-312, U. H.

1:00 - 3:00 Pathology-Surgery Conference; Auditorium Ancker Hospital.

1:00 - 2:50 Neurosurgery-Roentgenology Conference; W. T. Peyton, Harold O. Peterson and Staff; Todd Amphitheater, U. H.

4:00 - 5:00 Electrocardiographic Conference; George N. Aagaard; 106 Temp. Bldg., Hospital Court, U. H.

Saturday, June 4

7:45 - 8:50 Orthopedics Conference; Wallace H. Cole and Staff; Station 20, U. H.

8:30 - 9:30 Surgery Conference; Auditorium, Ancker Hospital.

8:00 - 9:00 Pediatric Psychiatric Rounds; Reynold Jensen; 6th Floor, West Wing, U. H.

8:00 - 9:00 Surgery Literature Conference; Clarence Dennis and Staff; Minneapolis General Hospital, Small Classroom.

9:00 - 9:50 Medicine Case Presentation; C. J. Watson and Staff; E-101, U. H.

9:00 - 10:30 Pediatric Grand Rounds; I. McQuarrie and Staff; Eustis Amph., U. H.

9:00 - 11:30 Surgery-Roentgenology Conference; Todd Amphitheater, U. H.

9:00 - 12:00 Neurology Conference; VA Hospital Annex, Fort Snelling.

10:00 - 11:50 Medicine Ward Rounds; C. J. Watson and Staff; E-221, U. H.

10:00 - 12:50 Obstetrics and Gynecology Grand Rounds; J. L. McKelvey and Staff; Station 44, U. H.

* *

SPECIAL LECTURE

Malignant Melanoma of the Skin...Dr. Lauren Ackerman
Washington University School of Medicine, St. Louis

8:00 P.M., Thursday, June 2, 1949, Medical Science Amph

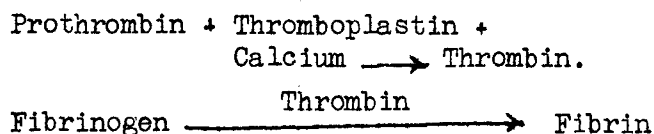
* *

II. BLOOD CLOTTING -- RECENT ADVANCES

R. Edward Bell

Investigations relating to the blood coagulation mechanism have been carried on over the centuries. The middle of the last century, however, marks the beginning of our present knowledge concerning this phenomenon. Since that time the studies can be roughly divided into three periods of activity. The first period was from 1850 to 1905, the second from 1905 to 1935, and the third from 1935 to the present time.

In the first period much of our fundamental knowledge of blood coagulation was established. It was during this time that the discoveries of thrombin and its precursor prothrombin as well as of fibrinogen and fibrin were made, and the role of calcium, thromboplastin and platelets demonstrated. Shortly after the turn of the century Morowitz, in Germany, assembled all this data together into a single theory which is now known as the classical theory of blood coagulation[1]:



Prothrombin, fibrinogen and calcium are normally present in the plasma. With the shedding of blood thromboplastin is released from tissue or from the platelets, activating the whole mechanism. The fibrin forms as insoluble protein strands and forms the structural framework of the clot. The blood is kept fluid in the vascular system due to the absence of free thromboplastin. As a fundamental concept the classical theory proved most valuable, and it is upon it that most present day theories are based.

During the next 30 years, from 1905 to 1935, little progress was made in our understanding of this phenomenon although much investigation was carried on. The discovery of heparin by Howell and his associates in 1918[2] and its subsequent isolation in pure form by Charles and Scott in 1933[3] stands out as the most significant contribution in this period.

In 1911 Howell formulated a theory based on the assumption that blood contained an anticoagulant or an inhibitor of clotting[4]. He thought that the presence of this anticoagulant substance rather than the lack of thromboplastin was responsible for maintaining the fluidity of the blood within the vascular system. The discovery of Heparin seven years later gave some support to the theory, heparin being incriminated as the Rey Anticoagulant substance. Although he had a large number of supporters in this country, most European investigators continued to support the classical theory. So much conflicting evidence and unjustified speculation appeared in the literature during this period that this interval is marked by confusion rather than by real progress. By 1935 the majority of workers had returned to the classical theory of Morowitz.

The modern period dates from 1935 onward and has been characterized by a great elevation in the clinical interest in blood coagulation, from a more or less passing interest in the relatively rare hemorrhagic diseases such as hemophilia, to a more fundamental interest in the mechanisms involved in blood clotting and bleeding disorders. This interest arose as a result of several discoveries which lifted the studies out of the rut in which they had so long been travelling. The most important of these was the discovery of Vitamin K and its vital role in prothrombin production. The great increase in the knowledge and importance of thromboembolic disease and the use of anticoagulants such as dicumarol and heparin as therapeutic agents has been a great stimulus also. The studies on Vitamin K and dicumarol have been very adequately reviewed elsewhere[5,6]. It is some other recent and interesting developments in the study of blood coagulation in relation to human disease that I wish to review here.

As these studies center principally

around prothrombin, it might be wise to consider briefly the methods currently in use in clinical and research laboratories for its estimation.

Prothrombin and Methods for Its Determination

There is not at the present time a satisfactory chemical method for measuring prothrombin described in the literature. All the methods so far described are biological ones, dependent upon the ability of prothrombin to form thrombin and the ability of thrombin to clot fibrinogen. They are generally classified into one stage methods and two stage methods, the one stage method exemplified by Quick's procedure[7] and the two stage by that of Warner, Brinkhouse, and Smith[8].

Quick's method which is the one in most common use is based on the assumption that if optimum amounts of thromboplastin and calcium are added to an oxalated plasma the time taken for the plasma to clot will bear a constant relation to the amount of prothrombin present. Let us refer once again to the classical theory upon which this is based. The time required for the usual concentrations of thrombin to clot fibrinogen is extremely small and is quite stable. That the time required for prothrombin to form thrombin is a measure only of the prothrombin concentration if optimum amounts of thromboplastin and calcium are present has not been demonstrated. Indeed it has been shown to be otherwise.

Quick further refined this test to measure the percentage concentration of prothrombin as compared with a normal in comparing the prothrombin time with the prothrombin times of saline dilutions of a normal plasma. A normal prothrombin time, that is 100 per cent concentration, by Quick's method is 12 seconds, as shown in Figure 1.

Warner, Brinkhouse and Smith, working at the University of Iowa, developed a two stage technique. In this method they carry out each half of the clotting mechanism separately. They first convert all of the prothrombin to thrombin with thromboplastin and calcium and then measure the thrombin produced separately by its activity on a standard fibrinogen solution. This is done by first removing the fibrinogen from the plasma, then with the addition of thromboplastin and calcium, allowing the first equation to progress for a period of three to seven minutes. This they believe allows enough time for all the prothrombin to be converted to thrombin. The amount of thrombin formed is then measured by adding an aliquot to a prepared fibrinogen solution. By dilution the amount of thrombin necessary to clot the standard fibrinogen solution in 15 seconds is determined. They have defined one unit of thrombin as that amount which will clot 1 cc. of standard fibrinogen solution in 15 seconds at 37°C. Since the reaction is stoichiometric the same units may be applied to prothrombin.

It can readily be seen that this method, theoretically at least, makes allowance for any influences capable of accelerating or slowing down the formation of thrombin from prothrombin. A normal human plasma contains about 300 units of prothrombin per cubic centimeter.

What is the correlation between the two methods? Fresh normal plasma will give comparable results. Stored plasmas and other abnormal plasmas give poor correlation. Table 1.[9]

The reason for the discrepancy has been rather heatedly discussed in the literature, and it has only been recently that the reason for it has become apparent. Several workers working quite independently have shown the presence in plasma of what has variously been called a component, a factor, or a globulin. In the presence of these substances the conversion of prothrombin to thrombin is greatly accelerated. Since the methods by which these investigators have demonstrated this factor have varied greatly, it is of interest to see how they arrived at the conclusions they have.

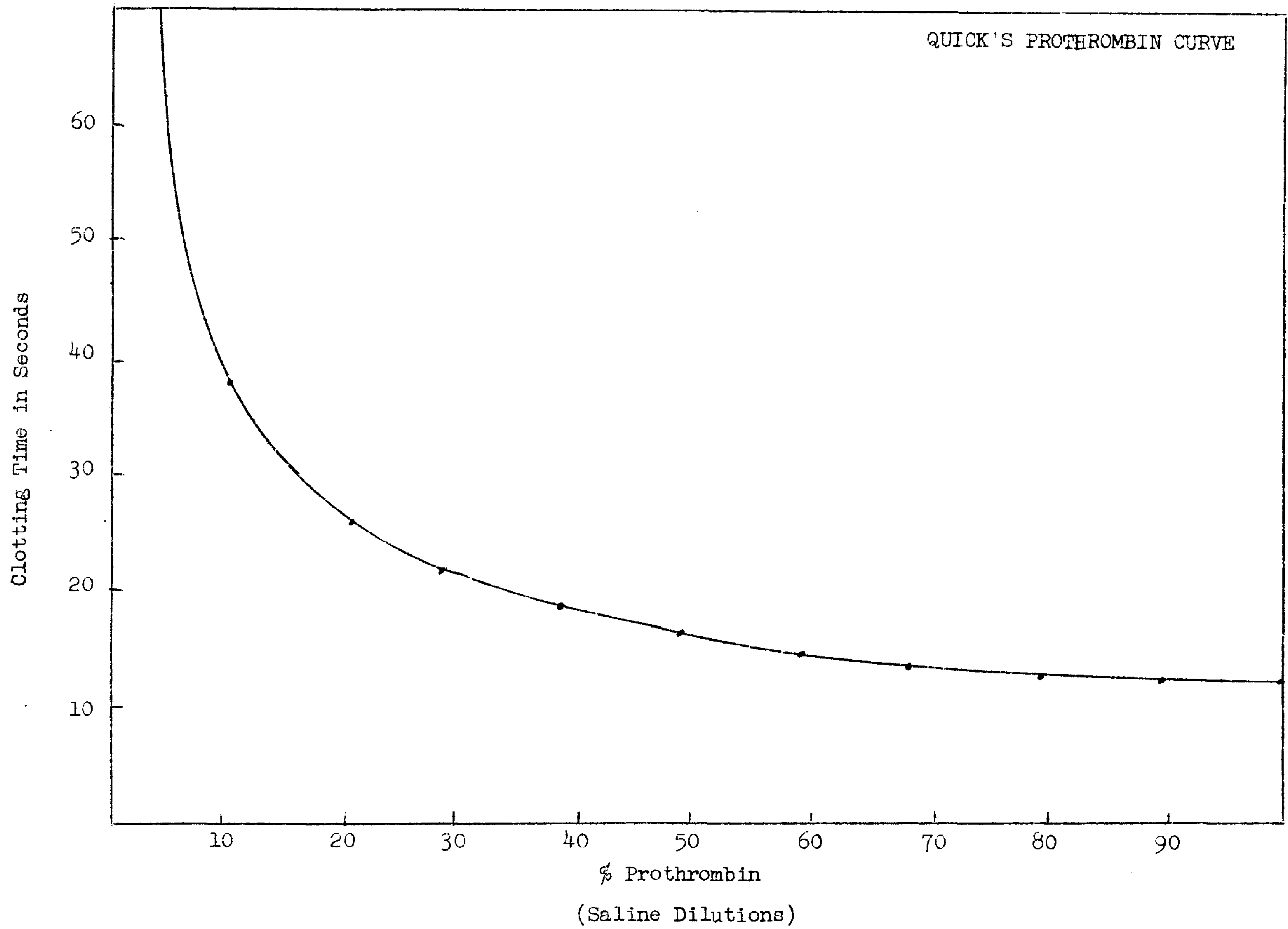


FIG. 1

Accelerator Factors

Bordet in 1912 and 1910 had put forward some evidence to the effect that prothrombin was normally present as a precursor and had to be activated by calcium before it could begin converting to thrombin[10]. Leggenhager had reported in 1936 and 1940 somewhat similar conclusions[10]. He demonstrated that the kinetics of the clotting of blood could not be explained on the basis of the classical theory. He showed a latent period between the initiation of the reaction and the time that any thrombin appeared and postulated that a preliminary reaction took place activating a "plasma thrombokinase" to throbokinase which then acted on prothrombin. Neither of these investigations received wide acceptance.

In 1946 Fantl and Nance[11] reported the presence of an accelerator of coagulation that was present in prothrombin free plasmas. Their investigations were done to determine the cause of discrepancies they noted between the undiluted and diluted one stage prothrombin methods. By plotting dilution curves using saline and prothrombin free plasma respectively they showed the presence of a much greater activity in the plasmas diluted with prothrombin free plasma. On the basis of this observation they reported the presence of an accelerator of coagulation which was not prothrombin.

Owren, working in Norway during the war, put the concept of this hitherto unknown factor in blood coagulation on a firm basis[10,12].

In 1943 Owren had under his care at the University Hospital in Oslo a 29 year old woman who gave a history of numerous severe bleeding episodes, the first of which occurred as severe epistaxis at $3\frac{1}{2}$ years of age. Over the years she had had spontaneous hemorrhages into her vitreous humor leaving her almost blind, many massive subcutaneous hemorrhages as a result of minor trauma and severe hemorrhagia which confined her to bed during her menstrual periods and on one occasion necessitated blood transfusion. The blood transfusion was noted to have had a rapid hemostatic effect. She gave no family history of bleeders, and her parents and eight brothers and sisters were all well.

Laboratory studies done on this patient showed a prolonged Lee White clotting time of 25 minutes compared with a control of 6 to 10 minutes. The platelet count was 400,000. Quick prothrombin times were 70 to 80 seconds compared with controls of 15 to 20 seconds. Clot retraction, plasma fibrinogen concentration, and serum calcium were all within normal limits. Liver function studies failed to reveal any evidence of dysfunction. Massive doses of Vitamin K had no effect on the prothrombin time or the bleeding tendency. No cause for the prolonged prothrombin time could be found. The presence of an anti-coagulant could not be demonstrated.

It has been well established that prothrombin can be quantitatively removed from plasma by the use of adsorbents such as $Mg(OH)_2$, $Al(OH)_3$, $BaSO_4$, etc.)[13]. Owren applied this adsorption technique to some of the patient's plasma and to normal human, guinea pig and ox plasma. He then made dilutions of the patient's plasma with these prothrombin free plasmas. The results are illustrated in Fig. 2.

Even minute amounts of normal human or animal prothrombin free plasma would greatly reduce the prothrombin time of the patient's plasma. Prothrombin free plasma from the patient had almost no effect. In other words, by adding very small amounts of something in plasma other than prothrombin he was able to reduce the prolonged prothrombin time to normal. This "something" was not present in the patient's plasma, and the evidence indicated that it could not be prothrombin.

As it was not thromboplastin, prothrombin, calcium or fibrinogen that was lacking and had not previously been described, he named it Factor V. The hemorrhagic disease resulting from a

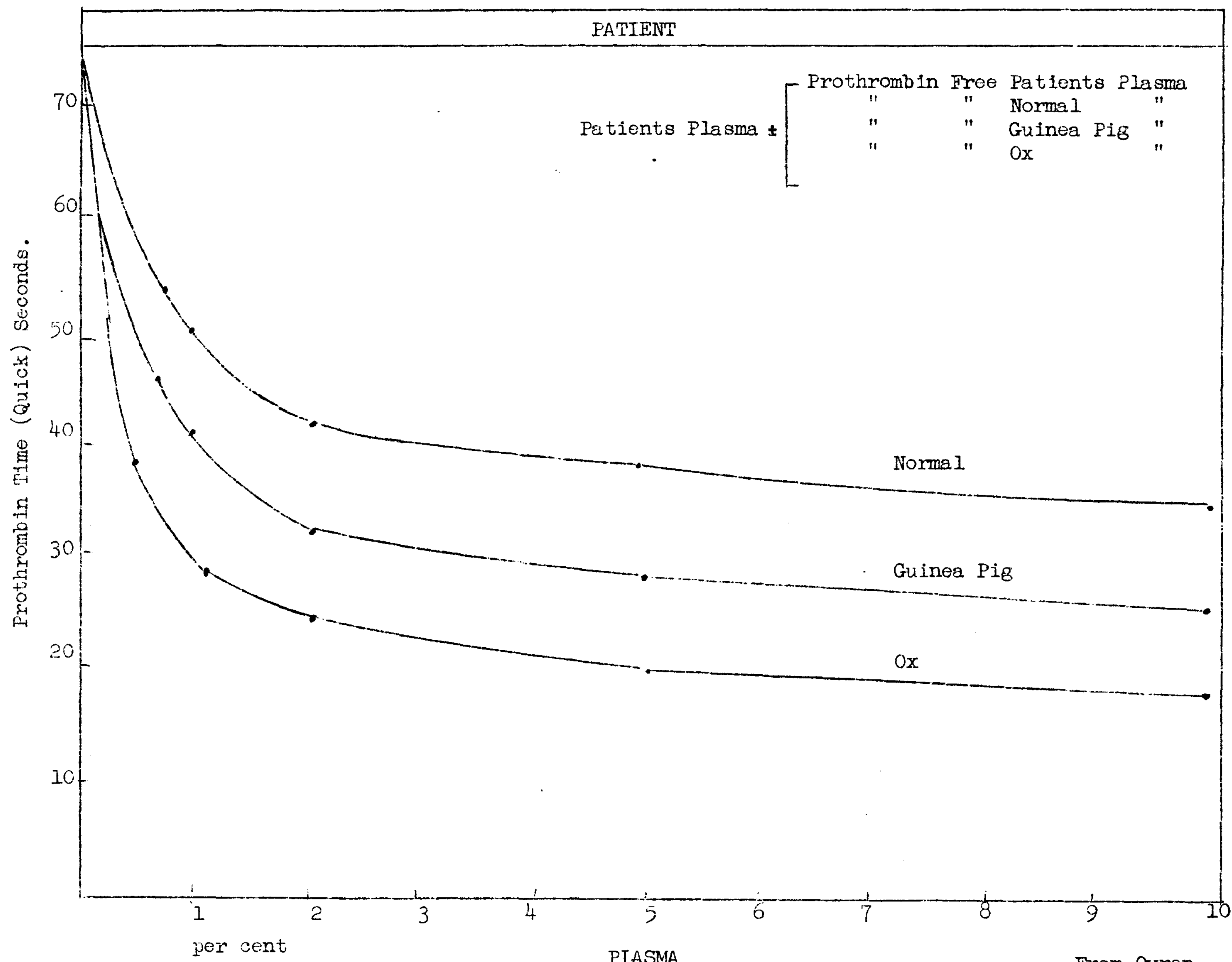


FIG. 2

From Owren

lack of this factor he termed parahemophilia.

Owren has investigated this girl very thoroughly and studied the Factor V he has described in considerable detail. The essential findings are shown in charts 1 and 2. From 11 cc. of normal human plasma he was able to prepare a concentrate which when injected intravenously would reduce the prothrombin time of the patient from 65 seconds to 28 seconds.

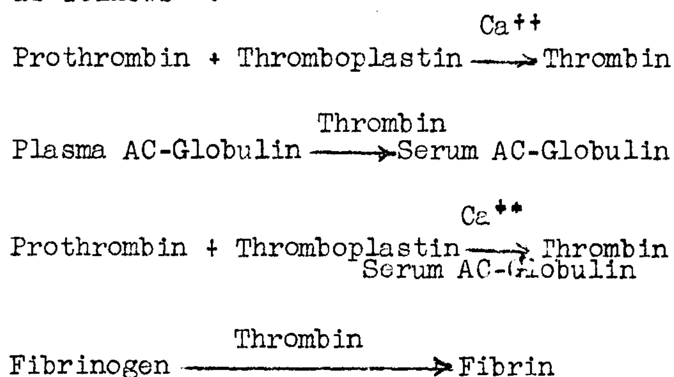
Factor V is a protein and exists in plasma in an inactive form. It must be activated by calcium and thromboplastin to an active form which Owren calls factor VI. Factor VI then affects the clotting mechanism. In the absence of prothrombin these factors will not form thrombin. He also has called factor V and factor VI pro prothrombokinase and prothrombokinase, respectively.

Seegers and his co-workers at Wayne University have described a similar new factor involved in the coagulation process. Seegers is a chemist and has been interested in obtaining pure plasma fractions of the various components that make up the clotting mechanism. His attention was focused on prothrombin and in 1948 he prepared a concentrated preparation of prothrombin of high activity[14]. His method was based on adsorption of the prothrombin on to $Mg(OH)_2$ with subsequent elution by CO_2 under pressure. These preparations have an activity of about 1500 units per mgm. of dry weight[15] which is about 500 times the concentration normally present in plasma protein. This prothrombin is a glycoprotein with a molecular weight of about 140,000. In solution it will very slowly convert to thrombin without the addition of either thromboplastin or calcium[15,16] but the presence of these two materials greatly accelerates the conversion. A bovine preparation which is converted to thrombin, lyophylized and sealed in ampoules is available commercially as Thrombin-Topical (Parke Davis Co., Detroit, Michigan).

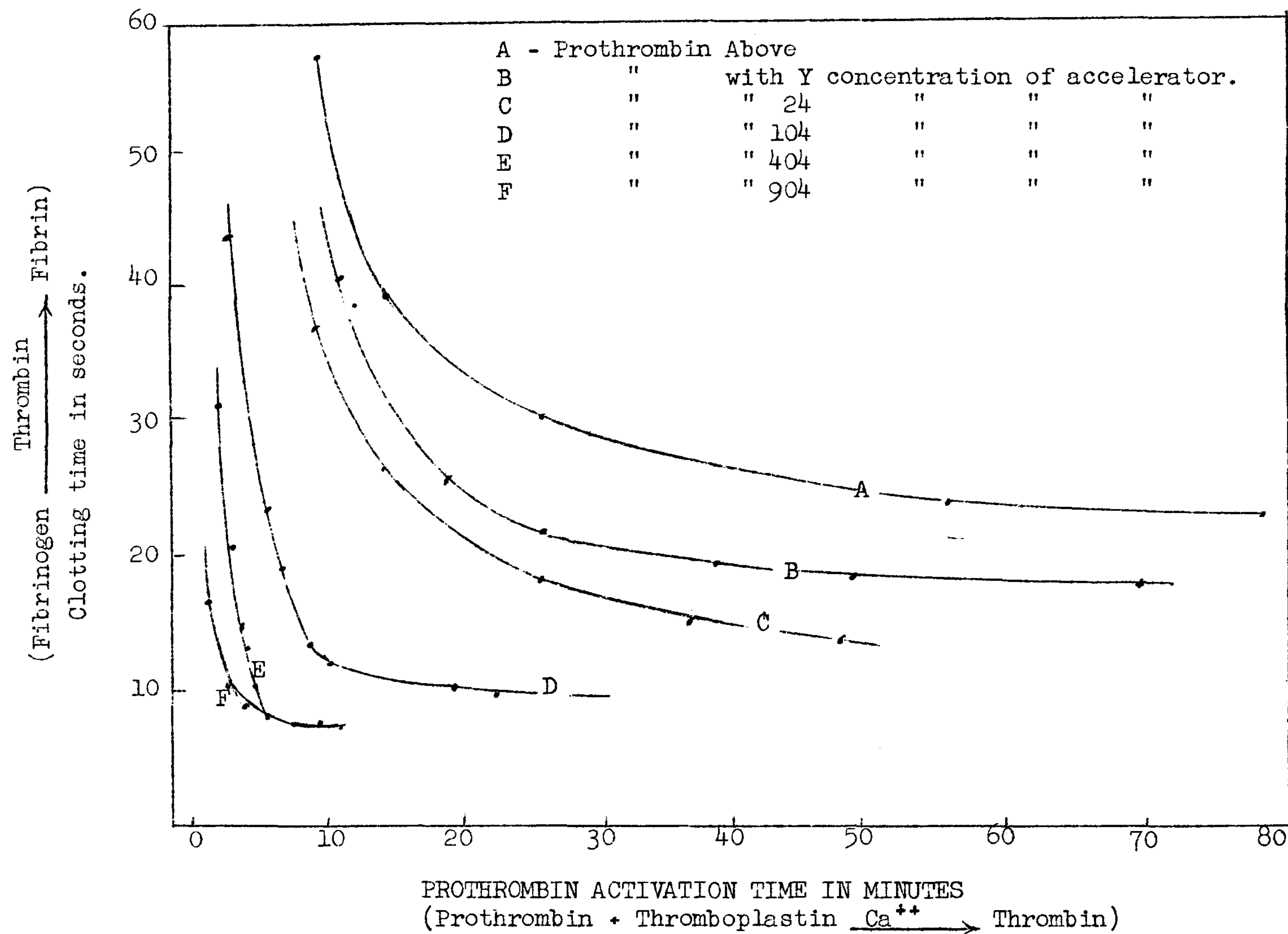
When Seegers compared his concentrated preparation with plasma prothrombin in its native state in plasma, he found his preparations were much less active[17]. By retracing some of the steps in his isolation procedure and analyzing some of the by-products, he was able to demonstrate a protein that greatly accelerated the conversion of his pure preparations. By adding very small amounts of the protein he could increase the activity of his pure preparation of prothrombin so that its performance equalled that of a similar amount of prothrombin in plasma. This effect is illustrated in Fig. 3. This factor, which he first reported in 1947, was named accelerator globulin and abbreviated to AC-globulin. Seegers and his group have investigated this factor in some detail and the more important characteristics are shown in Charts I and II.

When AC-globulin is isolated from plasma, it has considerably less activity than when it is isolated from serum[18]. By adding minute amounts of thrombin to AC-globulin from plasma its activity is stepped up to equal that of AC-globulin isolated from Serum. These are called plasma AC-globulin and Serum AC-globulin respectively and are distinguishable on the basis of their activity[18].

The part played in blood coagulation by these accelerators is believed to be as follows[18]:



Stored human plasma develops a prolonged prothrombin time when the determinations are done by Quick's (one-stage) method.



Activation of purified prothrombin with optimum amount of thromboplastin and calcium. Only AC Globulin concentration was varied. (From Ware, Guest and Seegers 17)
Curve F is virtually the same as a native prothrombin curve.

FIG. 3

Quick in 1943 observed that the prothrombin time of stored oxalated human plasma could be restored to normal by the addition to it of fresh dog or rabbit plasma heated with $Al(OH)_3$ that is, prothrombin free plasma.[19] The prothrombin time could also be restored to normal by the addition of plasma from a dog markedly hypoprothrombinemic as a result of dicumarol therapy. To explain these observations he postulated that there were in reality 2 components which went to form prothrombin. One of these, Component B, was deficient in dicumarol therapy and in $Al(OH)_3$ adsorbed plasma. The other, then called Compound A, was not affected by dicumarol therapy nor was it adsorbed on $Al(OH)_3$ but was lost when oxalated plasma was stored.

In 1947 Quick modified this view somewhat in consequence of studying 2 cases of idiopathic hypoprothrombinemia[20]. He has reported the results of studies done on the members of 2 families. In one family the mother, one son and one daughter have constantly shown prolonged Quick prothrombin times varying from $15\frac{1}{2}$ to 16 seconds compared with a control of 12 seconds (i.e., 50 to 60% concentration by Quick's curve). In the second family are 2 boys who have been bleeders from birth and who show a prothrombin time of 19 seconds (i.e., 35% concentration), constantly. (Tables 2 and 3).

From the data shown, by mixing these pathological plasmas with equal parts of dicumarol plasma, or stored plasma or adsorbed plasma and then determining the prothrombin time of the mixture he shows the following results. Both patients plasmas if fresh will restore stored plasma to near normal. One patient's plasma (R.F.) is similar in its defect to the plasma of a dicumarol treated patient. The other patient is not. A mixture of equal parts of both patients' plasmas results in a plasma with a normal prothrombin time. Since neither patient appears to have the deficiency found in stored plasma and each patient appears to have a deficiency different from the other he postulates that there are 3 different factors involved, i.e., a labile factor and two others.

Quick has changed his previous terminology[20] somewhat and has named his original component A the "labile factor" and has given the name Component A to the new factor apparently lacking in Vitamin K deficiency. He has then, three components which he says form a pro thrombin complex, A labile factor which is lost on storage and is not adsorbed on the usual adsorbants of prothrombin, a component A in the synthesis of which Vitamin K is essential and a component B the formation of which is suppressed in dicumarol therapy.

Since the original reports there have been a number of publications describing somewhat similar factors.

Milstone[21] at Yale University has described a 3 stage method of blood coagulation based on the theory presented by Leggenhager[21]. This method he uses to determine the presence and amount of a plasma prothrombin accelerator which he calls prothrombokinase. Some details of the characteristics of this accelerator can be seen in Charts I and II.

De Vries, Alexander, and Goldstein have described a Serum Prothrombin Conversion Accelerator, a name which they abbreviate to the letters S.P.C.A.[22]. They are unwilling at the present time to identify this as the same globulin as that described by Seegers or Owren. Its characteristics are shown in Charts I and II.

Honorato working with Quick has described what he terms the Plasmatic Cofactor of Thromboplastin[23]. He states that it does not belong to prothrombin and is necessary for the action of thromboplastin. It appears to be identical to Quick's labile factor.

Charts I and II summarize the chemical and physical properties of these various factors as they have been described by the original authors. It can be seen that there is a great similarity as re-

TABLE 2. Prothrombin Defect in subject R.F. (family 1)

	TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
	Fresh normal		12
	Stored normal (5 days old)		32
	R.F. fresh		16
	R.F. stored (2 days old)		24
I	R.F. fresh	1 volume	$13\frac{1}{2}$
	Normal fresh	1 volume	
II	R.F. fresh	1 volume	16
	R.F. stored	1 volume	
III	R.F. fresh	1 volume	$13\frac{1}{2}$
	Normal stored	1 volume	
IV	R.F. stored	1 volume	$13\frac{1}{2}$
	Normal stored	1 volume	
V	R.F. stored	9 volumes	16
	Dog $Ca_3(PO_4)_2$ treated	1 volume	

gards their fundamental activity on the coagulation mechanism. They have not been studied in complete enough detail to include them definitely as one protein. This possibility has not been entirely excluded however. They are all proteins and are thus difficult to study and classify except by arbitrary physical and chemical standards. Electrophoresis or Cohn's fractionation is of little value since the concentration of these factors is so small. In as far as the investigators themselves are concerned most feel that their particular factor is the same or at least closely akin to Owren's factors V and VI.

It is generally felt that these new accelerator factors do explain the discrepancy between the one stage and two stage methods of prothrombin analysis. A deficiency of accelerator factor will greatly prolong a one stage prothrombin time but may allow a normal "prothrombin concentration" by the two stage method because of the additional time allowed for the conversion of prothrombin to thrombin in the latter. The two stage test appears to be the most accurate measure of prothrombin while the one stage test is a summation of the activities of several factors, one of which is prothrombin.

What are the methods for the determination of these accelerator factors? At the present time there is no standardized procedure, and each investigator has his own method. They all depend on the ability of prothrombin free plasma to accelerate the conversion of pro-

TABLE 3. Prothrombin defect in subject B.B. (family 2)

TYPE OF PLASMA		PROTHROMBIN TIME IN SECONDS
Fresh normal		12
Stored normal (2 days old)		19
B. B. fresh		19
B. B. stored (2 days old)		24
I B. B. fresh Normal fresh	1 volume 1 volume	12
II B. B. stored Normal fresh	1 volume 1 volume	12
III B. B. fresh Normal stored	1 volume 1 volume	$11\frac{1}{2}$
IV B. B. fresh Dog $Ca_3 (PO_4)_2$ treated	9 volumes 1 volume	$19\frac{1}{2}$
V B. B. stored Dog $Ca_3 (PO_4)_2$ treated	9 volumes 1 volume	19
VI B. B. fresh Human fresh $Ca_3 (PO_4)_2$ treated	1 volume 1 volume	29
VII B. B. fresh Physiological saline	1 volume 1 volume	29
R. F. Fresh plasma 1 volume B. B. Fresh plasma 1 volume		$13\frac{1}{2}$ seconds

Chart I

AUTHOR	DESIGNATION	TYPE OF STUDY
Owren	Factor V	Detailed study of a patient with a peculiar hemorrhagic tendency. Showed: 1. Prolonged Quick prothrombin time and clotting time. 2. Normal prothrombin content. 3. Addition of minute amounts of normal plasma invitro restored prothrombin time to normal. 4. Administration of normal plasma or a concentrate of normal plasma to patient corrected temporarily coag. defect.
Seegers	AC-Globulin	Concentration of proteins involved in clotting mechanism. Confirmed Owren's finding of an additional factor concerned in conversion of Prothrombin to Thrombin. Analysis of Plasma and Serum shows the latter to contain the accelerator in a more active form than found in plasma.
Milstone	Prothromlokinase	Study of Leggenhager's theory of Blood Coagulation showed clotting occurs in three stages. The first stage is an activation of a plasma "Kinase" which then initiates and accelerates the conversion of Prothrombin to Thrombin.
Quick	Component A Component B Labile factor (originally Component A)	Studies on stored plasma and on patients with familial by poprothrombinemia by one stage method. Prothrombin a complex of these components.
Honorato	Plasmatic Cofactor of Thromboplastin	Studying the adsorption of prothrombin by $Ca_3(PO_4)_2$, $Al(OH)_3$ etc. A factor separated that is not prothrombin but aids prothrombin conversion to thrombin.
Fantl and Nance	Plasma factor	Prothrombin time of plasma diluted with prothrombin free plasma is less than prothrombin time of plasma diluted to an equal degree with saline. First report of this phenomenon.
Alexander	S.P.C.A.	Small amount of serum added to plasma will result in a much reduced prothrombin time. Serum Prothrombin Conversion Accelerator.

Chart II

AUTHOR	TERMINOLOGY	METHOD OF DEMONSTRATION	PROPERTIES	REMARKS
Owren Acta. Med. Scand. 1947 Supp. 194	Factor V. Proprothrombo- kinase Factor VI Prothrombokinase.	"Parahemophilia"-prolonged Quick P.T. corrected by prothrombin free ($BaSO_4$ adsorbed) plasma. Isolated factor in crude form given to patient restored proth. time. Measurement-Effect of diluted plasma on mixture of Proth, Tpl-Fibrinogen. Incubate 3-4 min. then add $CaCl_2$.(i.e.convert Fact V→ Fact VI)	Moderately labile 60-70%. Left in 8 days. Heat to 57°-inactivate " " 37°-No inactivation. In plasma-ph 5.3 ppt. along with Proth. Ph range 4-9.5-inactivated beyond. Soluble-ppt.from soln. at ph 6.0. Will not dialyse. Ppt. from plasma with $(NH_4)_2SO_4$ 50% also with acid precipitation. Adsorption-partly on $Mg(OH)_2$, $Al(OH)_3$ & $Ca_3(PO_4)_2$	Fact V + Prothrombin Cytokinase(Ca^{++} → Fact VI Prothrombin Ca^{++} Fact VI → Thrombin. Fibrinogen Thrombin → Fibrin. Fact.VI-not isolated. Believe it to be same as: Quick labile. Seegers AC Glob. Fantl & Nance factor.
Seegers et al Assorted Publications-see J.B.C.- 169:231:1947	AC Globulin	Preparation of pure Proth. preparations. Found pure prep not as active as whole plasma. Analysis of by products of prep showed protein that restored the activity. Measure by its activity in accelerating the activation of a proth.soln. to thrombin. Prep.-ppt.from dil acidified plasma. Redissolve in Oral Saline & adsorb on $Mg(OH)_2$. Elute with CO_2. Fractionate in cold conc.$(NH_2)_2SO_4$. Dialyze & ppt at 150 elect pt.	Stability varies with Species. Series AC G. unstable in man-Stable in Bovine serum. More unstable in oxal.plasma. Plasma AG→ Serum AC Glob. Present in platelets also (centrifuge studies) Conc.Bovine 150 u/cc. Dog 150-200 u/cc. G Pig 30-40 u/cc. Man 12-17 u/cc. Destroyed by heat 53°x 2 hrs. Ppt. at ph 5.4 Not adsorbed by $BaCO_3$. Sensisitive to alkaline ph.	Proth Tpl + Ca → Platelet AC Glob. Thrombin. Plasma Thrombin → Serum AC Glob AC Glob. Proth Tpl + Ca Thrombin. Serum ACG. Fibrinogen Thromb → Fibrin. Believe it to be same as Owren Factors. Not same as Quick labile.
Milstone J.Gen.Physiol: 31:301-324:1948. Science 106: 546-47:1947.	Prothrombokinase	On literature evidence-3 stages of Blood Coag. Meas.1.Ca+Prothrombokinase at intervals. 2.Samples added to Prothrombin-incubate 2 min.	Heat labile 60° Cx 10 min.-marked loss. Ppt. at ph 5.2-5.5	$CaCl_2$+Prothrombokinase+ Prothrombin→ a latent period then a period of accelerated proth production. Prothrombin +Ca-incubate-

AUTHOR	TERMINOLOGY	METHOD OF DEMONSTRATION	PROPERTIES	REMARKS
Milstone (cont.)		3-Assay thrombin formed by clotting fibrinogen. Prepare-Remove Fibrinogen by heat 51°C.x16 min. Remove proth.by adsorption with $BaSO_4$. Dilute & ppt.at ph 5.2-5.5.		then add to prothrombin-no latent period. Prothrombinase $\xrightarrow{Tpl \quad Ca}$ Thrombokinase. Proth. $\xrightarrow[Ca]{Thrombokinase}$ Thrombin Fibrinogen $\xrightarrow{Thrombin}$ Fibrin Same as: Quick labile & Proth A Owren Fact V.
Quick Am.J.Physiol 150:381:1947	Labile Factor Component A Component B	Studies on stored plasmas with Quick method, and observations on 2 cases of idiopathic hypoprothrombinemia.	Labile Factor not adsorbed on $Al(OH)_3$. 0.0/m Comp.B-adsorbed by $Ca_3(PO_4)_2$. Comp.B-lacking in dicumarol Comp.A-lacking in Vit.K deficiency.	Labile factor-Dog plasma 10x human. Rabbit " 50x human Believes labile factor is Owren Factor V.Not Seegers factor.
Honorato Am.J.Physiol. 150:381:1947	Plasmatic Cofactor of Thromboplastin	Invest.adsorption of Proth. with $Ca_3(PO_4)_2$ & $Al(OH)_3$.	Non dialysable Adsorbed on 0.2 m $Al(OH)_3$ Lost when plasma stored	Labile Factor of Quick?
Fantl & Nance Aust.J.Sci. 9:117:1946 Nature 158:708 1946	Plasma factor	1.Plasma dil with Saline → longer P.T. than diluted with Prothromb.free plasma. 2.Proth + Fibrinogen +Tpl+ Ca → longer P.T. than proth + proth free plasma + Tpl + Ca.	Proth free plasma -Not adsorb with $Al(OH)_3$ or $BaCO_3$	Same as findings with $BaSO_4$ adsorbed plasma.
B.Alexander A.de Vries et al. Blood:4:247: 1949	S.P.C.A. Serum Prothrombin. Conversion Accelerator	Addition of Oxal serum to oxal plasma + $BaSO_4$ plasma shortens the P.T. measure by effect of serum on mixture of Oxal plasma + $BaSO_4$ plasma in lowering the P.T. of the mixture.	Not thrombin. Lost in stored plasma-requires a bile factor for its activation or max. effect. Not affected by Heparin. Heat labile 56° C. x 2.5 min = 10% left. Adsorbed on Seitz filter. Ph 4.5 to 8. Not dialysable. Not ppt. at ph 5.8 sensitive to alkalin p.h.	Dog greater than human. Not labile factor. May be Owrens Fact VI or Seegers Serum acceleration. Unable to produce SPCA from plasma by adding thrombin.

Beef Plasma Prothrombin Determinations

Day	Assay		
	Two Stage	Quick	
		Clotting Time	Activity
	u/ml	sec	0/0
0	190	16	100
1	190	18	67
2	190	19	62
3	188	23	42
4	190	26	33
5	192	33	23
6	190	42	13
7	188	52	10
8	185	63	8

From Loomis & Seegers[9]

Comparison of one stage and two stage methods as applied to stored plasma.

TABLE 1

thrombin to thrombin, however.

The simplest but least specific is that of Quick whereby the unknown plasma is mixed with stored plasma, dicumarol plasma or $BaSO_4$ adsorbed plasma and the prothrombin time of the mixture obtained[20]. By a comparison of the various prothrombin times so obtained a deduction as to the missing component can be made. This is not a quantitative procedure.

Seegers and his associates measure AC-Globulin activity by observing the effect of greatly diluted plasma on the conversion of pure prothrombin solutions to thrombin[18]. The limitations of this method are in part those imposed by the difficulties of obtaining pure prothrombin and fibrinogen preparations. It is, however, a very sensitive method. Owren uses a somewhat similar method, using pure prothrombin solutions[10].

Pathological Variations in Accelerator Factors

It has been conclusively established that prothrombin is formed in the liver and that Vitamin K is necessary for its elaboration. Since liver injury and dicumarol treatment depress the formation of prothrombin, what effect do they have on the accelerator factors? The best studies so far reported are those from Seeger's group.

Acute hepatic damage due to chloroform intoxication in dogs is followed by a pronounced fall in AC-Globulin activity and also by a fall in the prothrombin concentration to zero. On recovery the acceleration factor returns to normal somewhat faster than the prothrombin concentration[24].

Dicumarol administration to animals and humans will greatly reduce the prothrombin concentration of the plasma. The accelerator factor is only mildly affected with a reduction to approximately one half normal when the prothrombin congentration has dropped to one per cent[25]. On withdrawal of the drug an over-compensation occurs resulting in a rise in AC-Globulin activity up to over one hundred per cent of normal. If the drug is continued over a long period of time the AC-Globulin will return to normal in about three weeks irregardless of the fact that the dosage is sufficiently high to keep the prothrombin at a very low level. Dicumarol therefore has only a transient effect on the accelerator factor.

Owren's studies[26] of factor V have shown that it can be reduced to 50% of normal without producing a demonstrable bleeding tendency. In hepatitis where the prothrombin concentration is low, Factor V may be low also but is not always decreased; a progressive decrease in Factor V during the course of the disease indicates a poor prognosis. In obstructive jaundice it is increased in concentration whereas the prothrombin concentration tends to be decreased. Owren believes that continued low levels of Factor V indicates permanent liver damage. Dicumarol, he states, has no effect on Factor V.

In pernicious anemia although the level is normal in relapse it rises above normal with remission, the rise occurring before the reticulocyte response. Studies of cases with coronary thrombosis indicate that often a high value for Factor V is obtained but this is by no means a constant finding. In venous thrombosis in the acute stage Factor V concentration is almost always increased.

Clinical studies of any extent have not been reported by the other investigators.

A spontaneous reduction in these accelerator factors or a congenital lack of them occurs only rarely. Owren's case is really the only adequately studied one in the literature. A number of cases of idiopathic hypoprothrombinemia have been studied, one of which was studied at the University Hospitals by Dr. Paul Hagen and Dr. C. J. Watson[27] In reporting this case they have completely reviewed the literature on the disease. A deficiency of accelerator factors was excluded in their case. Some cases heretofore diagnosed as hypoprothrombinemia may, however, be deficiencies of accelerator factor as not all of them have been studied in complete enough detail to exclude this possibility.

The cases described by Quick, on whose findings he established his prothrombin complex theory, remain a puzzle. The studies so far reported do not reveal any data which can be used to correlate them satisfactorily with deficiencies of the prothrombin accelerators.

Jacques at the University of Saskatchewan has recently reported experimental studies on a new anti coagulant drug called 2 phenylindane dione 2,3.[28] He abbreviates this name to the letters P.I.D. When administered to rabbits in a dose of 8.3 mgm./K_9/8 hrs., it increases the prothrombin time by the one stage method to apparent infinity and a dose of 1 mgm./K_9/8 hrs. will double the prothrombin time. On withdrawal of the drug the prothrombin time returns to normal within 36 hours. Administration of Vitamin K does not accelerate this recovery. The experimental data indicate that it affects the activity of Factor V reducing it markedly without however affecting the quantity of this factor in the plasma. The methods by which this was established have not been reported as yet. The data would suggest

the presence of an inhibition of accelerator factors.

Hyperheparinemia

After Howell had isolated heparin from the liver many unsuccessful attempts were made to show its presence in normal blood. In 1938 Welander isolated heparin from the blood of dogs which were in peptone shock[29]. Three years later Jacques and Waters were able to isolate heparin from dogs in anaphylactic shock and they showed further that the heparin came from the liver[30].

A year ago Allen and his associates at the University of Chicago reported on studies on animals exposed to ionizing radiation[31]. It has been recognized for a long time that animals would develop a hemorrhagic tendency under such treatment. This had previously been attributed to the thrombocytopenia which also occurs. These workers observed that the animals developed a markedly prolonged clotting time before the platelet count fell or before the platelets show any other changes and frequently the animals developed hemorrhages with a normal platelet count. They were able to demonstrate the presence of an anticoagulant in the blood of these animals, and since heparin is the only known naturally occurring endogenous anticoagulant, they suspected it might be the cause of this hemorrhagic tendency.

Heparin is an acid compound being a heterogeneous group of sulphuric acid esters of mucopoly saccharides[32]. Its activity can be neutralized by certain basic compounds such as protamine sulphate or basic dyes such as toluidinic blue[32,33].

In vitro experiments showed that the clotting time of the blood of these animals could be reduced to normal by the use of these compounds and the injection of comparable amounts intravenously had a similar effect. This gave further indication that the anticoagulant might be heparin, and by applying to the blood a method that is used to isolate heparin from biological material they obtained an amorphous material with strong anti-coagulant properties which showed some of the characteristics of heparin. It is their belief that the bleeding resulting from experimental x-ray irradiation is probably due to hyperheparinemia.

The hematological findings after total body irradiation include a severe thrombocytopenia prolonged bleeding time and clotting time, purpura and an anemia. Aside from the prolonged clotting time these findings are similar to those of primary or secondary thrombocytopenic purpura. These investigators, therefore, carried their investigations over to the study of these disorders.

To determine small amount of heparin in the blood they devised a protamine tolerance test[34]. Since these patients do not have a prlonged clotting time as a rule, 10 cc. of blood is drawn into a standard amount of heparin to prolong the clotting time. One cubic centimeter amounts of this heparinized blood are then added to a series of ten test tubes which contain increasing amounts of protamine sulphate in normal saline. The clotting time is then determined on each tube. The amount of protamine necessary to reduce the clotting time to normal is used as a presumptive measure of the heparin that was neutralized. By comparison with a normal blood treated in a similar manner the amount of heparin in excess of that added is calculated.

Smith and his coworkers have presented evidence to show the presence of a heparin like anti-coagulant in the blood of patients treated with nitrogen mustard. This seems to be the first convincing demonstration of such an occurrence in man.

The administration of toluidine blue or protamine sulphate to patients with thrombocytopenia with an abnormal protamine tolerance test will often correct the abnormal result. Although patients

with thrombocytopenia often show an abnormal protamine tolerance, this is found also in hypoprothrombinemia and hemophilia; or any condition which will prolong the whole blood clotting time. The test as described cannot be considered a test for heparin level in the blood[36]. Some of the effect of the intravenous toluidine blue or protamine sulphate has probably been due to a vascular effect as one of the most prominent clinical effects with their use is a decrease in petectrial. The clinical results with the use of toluidine blue or Protamine Sulphate in hemorrhagic diseases has been disappointing[37,38].

Jacques has recently described a much improved method for the isolation of heparin[39]. He has isolated a heparin-like compound from normal human blood and has been able to recover up to 60 to 80 per cent of heparin added to whole blood[40]. His figures indicate that the normal level of "heparin" in human blood is of the order of 0.009 mgm. per 100 ml. This method has not as yet been applied to clinical conditions. If this material proves to be heparin it is the first time it has been isolated from the normal blood.

Circulating Anti-Coagulants

Since first reported in 1940 by Lozner, Joliffe and Taylor the presence of a substance in the blood which would prolong the clotting time of a normal blood has been described by several authors[41,42,43,44]. Generally speaking, these anti-coagulants reveal themselves by precipitating atypical hemorrhagic disorders with laboratory findings of a prolonged clotting time. These disorders have been observed in both males and females. The exact nature of these anti-coagulants is unknown but they do not appear to be heparin-like materials.

One of these anti-coagulants has been found in hemophiles. Hemophilia is a hereditary hemorrhagic disease of males characterized by a prolonged clotting time and normal bleeding time. It is propagated from generation to generation by the female who however does not suffer from the disease herself. Although it was once thought that there was a primary defect of the platelets in these patients which prevented their breakdown to release thromboplastin it has now been shown that the defect is in a plasma factor[45,46]. Normal people have a globulin present in the plasma that causes platelets to rupture when blood is shed. The hemophiliac lacks this factor and therefore the platelets are not broken down readily and there is a delay in the release of thromboplastin. This results in a prolonged clotting time. The factor in plasma lacking in hemophilia is called anti-hemophiliac globulin and it has been obtained from human plasma in a highly concentrated form[45]. In Cohn's separation of plasma proteins it comes out in Fraction I and this fraction can be effectively used in treatment of hemophiliac bleeding[47]. This anti-hemophiliac globulin does not appear to be related in any way to the accelerator factors[48]. Some hemophiliacs who have received many transfusions or injections of anti-hemophilic globulin become resistant to treatment and develop anti-coagulant properties towards normal blood tested in vitro[49]. Craddock and Lawrence have studied such a case and have made some interesting observations regarding the nature of this anti-coagulant[50]. They believe that in these cases the difficulty is due to an antibody developed against the anti-hemophilic globulin administered. The anti-hemophilic globulin appears to act as an antigen in these cases. They have shown the presence of precipitins developed against normal blood and Cohn's fraction I containing the anti-hemophilic factor. They describe this antibody as an anti anti-hemophilic globulin.

Many of the cases of circulating anti-coagulants reported have not been hemophiliac and not all have given a history of previous transfusion. The exact nature of this phenomenon remains obscure at the present time.

Hypercoagulability

A discussion of recent advances in Blood Coagulation would not be complete

without some reference to the practical problem of the determination of hyper-coagulability. No one would question that a simple clinical method which would give reliable information regarding the tendency or the propensity for patients to develop intravascular thrombosis would be of inestimable value. Several attempts have been made to demonstrate hyper-coagulability.

Dr. Takats in 1943 developed a heparin tolerance test[51]. He gave patients a standard dose of 10 mgm of heparin and then followed their clotting time using the simple capillary tube method. If he failed to get an increase of over 4½ minutes in the clotting time in 10 minutes after the heparin was given he considered the patient to have an abnormal tolerance to heparin and an increased tendency to clot. He demonstrated resistance by the method occurring post operatively, following cerebrovascular accidents and in Buerger's disease.

Tuft and Rosenfield in 1947 modified this test and felt it was a reliable indication of a tendency for intravascular thrombosis[52].

Waugh and Ruddick developed a test whereby the clotting time was determined after adding graduated amounts of heparin to the blood in Vitro[53]. A poor response to the heparin, that is, a short clotting time compared with a normal blood containing the same amount of heparin, was taken as evidence of increased coagulability. They found the test positive in cases of severe infection, following hemorrhage, and post-operatively. A comparison of the in vitro and in vivo heparin tolerance tests, made by Hagedorn and Barker, showed good correlation between the 2 methods[54]. Silverman has recently reported a series of cases he has shown an increased tolerance to heparin beginning about 24 hours post-operatively and lasting for about a fortnight[55].

The use of the prothrombin time to determine hyper-coagulability has also been described but with considerably less consistent results. Shapiro used both 12.5% diluted plasma and undiluted[55,56] plasma. The difference between the two results was taken as a measure of prothrombin activity. A smaller difference than normal values was taken to mean either hyper-coagulability or the presence of an anti-coagulant. He concluded that a reactive hyper-prothrombinemia was a frequent accompaniment of developing thromboembolism[45]. Cotlove studied a series of cardiac patients and could not demonstrate good correlation between prothrombin time and thrombosis. The effects they did get they believed could be secondary from the thrombosis[57].

Tuft and Rosenfield carried out a very extensive evaluation of the accelerated dilute prothrombin time as an indication of hyper-coagulability[58]. They were unable to show any correlation between the laboratory results and the clinical findings of thrombosis. Mann and Hurn were unable to show any definite trend of prothrombin time or anti-thrombin level in venous thrombosis[59].

In 1948 Mahoney and Sandrock reported a series of cases which they followed with whole plasma prothrombin times and 25% dilution prothrombin times. According to the standards they have set up all of the cases, 16 out of 300 studied, who developed venous thrombosis had hyperprothrombinemia[60].

Also in 1948 Lyons reported that fibrinogen clots in 2 stages first forming, as a result of oxidation of its sulphydryl group, a fibrinogen B, and then a further oxidation forming fibrin[61]. The fibrinogen B is soluble and while not present normally in plasma appears when the blood has an abnormal tendency to coagulate. It can be demonstrated by adding a small amount of B naphthol and alcohol to oxalated plasma. If fibrinogen B is present it will form a white gel. The test is positive in cases which have pyogenic infection or venous stasis. No large series of cases of venous thrombosis have been reported to establish this test as a measure of

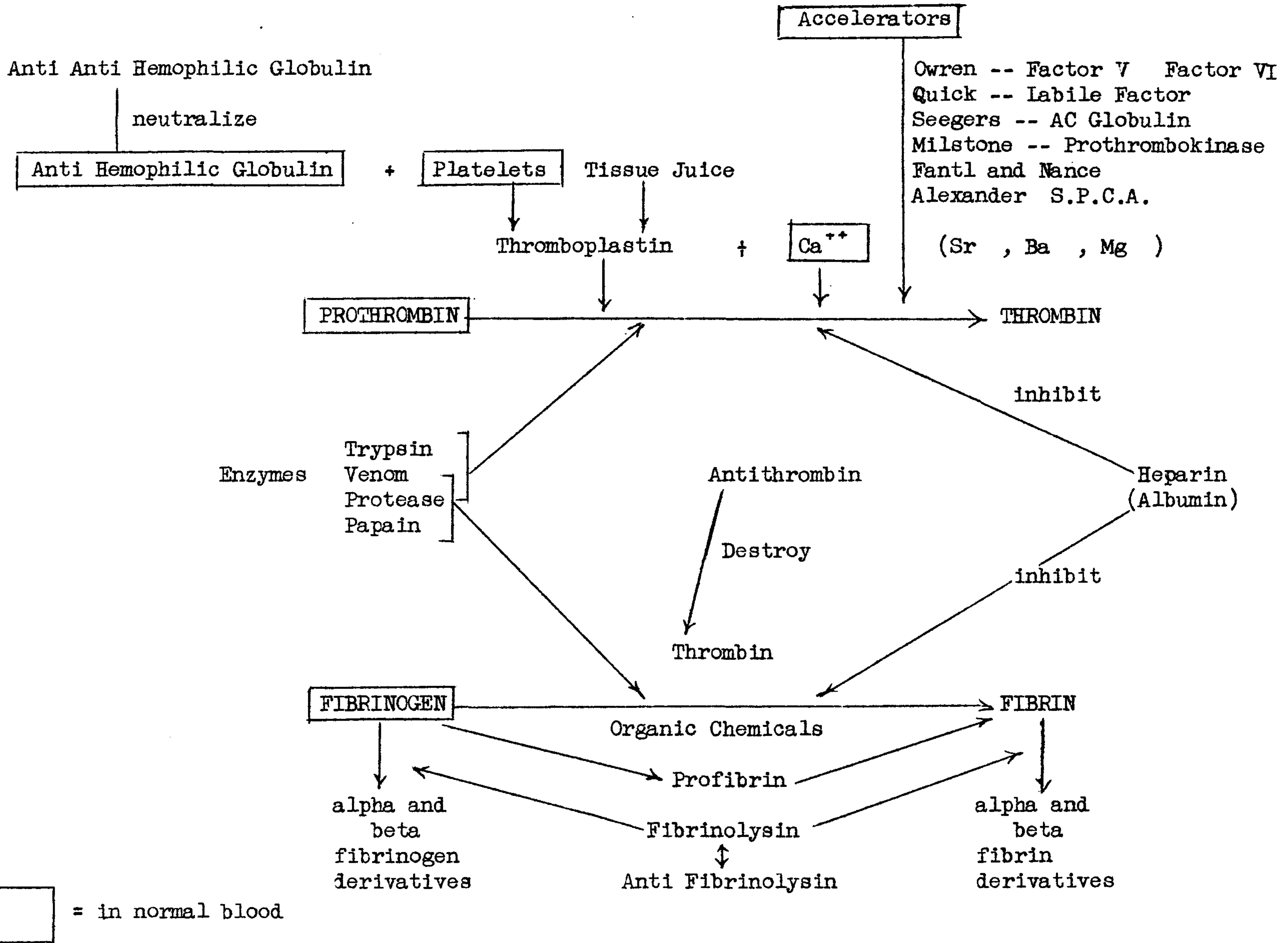


CHART III

hypercoagulability within the vascular systems.

No method of detection of an intravascular thrombosis tendency is firmly established although some of these methods merit further investigation. Whether or not the accelerator factors play a dominant role in thrombic disease has yet to be demonstrated.

It is impossible to touch even briefly on all the recent developments in the field of study. Important advances have been made in the solving of the exact nature of the hemostatic dysfunction in hemophilia and as already mentioned concentrated plasma fractions have been prepared to use in its treatment. As far as can be determined it has no relation to accelerator factors[48]. Studies on the enzyme fibrinolysin which is responsible for the dissolving of the clot have also been reported from many laboratories. Loomis has concentrated an anti fibrinolysin to 80 per cent purity and has described many of its characteristics[62]. Variations in the serum level of antifibrinolysin in various pathological states has been reported by Guest[63].

Fibrinogen has been obtained in almost a pure, that is, 100 per cent clottable state[64]. Many studies have been done on the nature of the fibrinogen to fibrin reaction and also on the nature of the fibrin gel and the physical chemistry that explains its special characteristics[65]. These developments are largely in the field of pure chemistry and at the moment have little clinical application.

Chart III is a schematic represetation of the many factors that influence the coagulation of the blood. Space does not permit a full description or even brief mention of all these substances. In the study of any bleeding disorder one must not neglect consideration of the integrity of the vascular system itself. Little is known of the physiology of the capillaries insofar as their relation to bleeding disorders is concerned. They undoubtedly are an important factor in many of the perplexing hemorrhagic diathesis encountered clinically.

<u>Summary</u>

A few of the more significant advances in the study of the mechanism of blood coagulation have been reviewed. The discovery and the characteristics of hitherto unknown factors which accelerate the coagulation of blood have been discussed in some detail and some of their variations in human disease have been described. Until better methods for their study are available which will allow their clear cut separation, final conclusions as to their identity should be made with caution.

Although the experimental evidence to show the presence of hyperheparinemia in x irradiation and possibly in Nitrogen Mustard Therapy is quite convincing, the presence of increased heparin in the blood in thrombocytopenic purpura and other hemorrhagic diseases has not been satisfactorily demonstrated.

The two stage method is the only sound method available for the determination of Prothrombin. The one stage method is a measure of the over all "clottability" of the plasma. Although it is affected by variations in the level of prothrombin it is also affected by other factors. In spite of its lack of specificity it remains probably the best method at the moment for the clinical control of dicumarol therapy.

Attempts to demonstrate an increased tendency to clot in impending intravascular thrombosis have so far proven unsuccessful. However recent reports appear to be more encouraging in this regard.

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III. MEDICAL SCHOOL NEWS

Faculty News

John K. Meinert, senior medical student, was honored at the University's annual Cap and Gown Day Convocation on May 12. He was presented with the annual award of the Southern Minnesota Medical Association. Each year this Association awards a medal and a prize of $100 to the most outstanding member of the senior class.

John Meinert had not only maintained an honor point average of 2.8 through his junior year, but had held various class offices and had manifested a genuine interest in the welfare of his classmates and the Medical School. Meinert is a member of the Phi Beta Phi medical fraternity and son of Dr. Albert E. Meinert, of Winona, Minnesota. He will begin his internship at the University of Minnesota Hospitals on July 1, 1949.

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Progress Note

Work is proceeding at an encouraging rate on the Variety Club Hospital. The work of excavating has been completed, footings are in, and concrete has already been poured which forms some portions of the basement walls. Some idea of the size and contour of the building can be obtained by the outline of the foundation. Workmen are almost ready to pour concrete which will form the basement floor of one section of the building. It is hoped that the building may be ready for dedicatory ceremonies in the fall of 1950.

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New Minn. Medical Foundation Members

L. H. Heinz, M.D., Wabasso
Woodard Colby, M.D., 809 Lowry Bldg., St. Paul

Biographical Briefs -- Chief of Surgery

O. H. Wangensteen was born in western Minnesota in the town of Lake Park. He attended high school in Lake Park and entered the University of Minnesota for his pre-medical work. In 1919 he received his Bachelor of Arts degree at the University of Minnesota. His undergraduate medical studies were also done at this University, and he received his M.D. degree in 1922. Dr. Wangensteen became a Fellow at the University of Minnesota Hospitals in 1923. The following year was spent as a Fellow in Surgery at the Mayo Clinic in Rochester. He returned to the University Hospitals as a resident in Surgery in 1925, and in that year received his Ph.D. degree in Surgery.

Dr. Wangensteen was appointed instructor in the Department of Surgery in 1926 and has been active in teaching and research in surgery here at the University since that time. However, he was away from our campus for one year in 1927 and 1928 when he studied abroad. This time was spent as an assistant in the clinic of Professor F. de Quervain at Berne, Switzerland.

He was made an Associate Professor in 1928, and in 1930 was appointed to the position of Surgeon-in-Chief at the University Hospitals. His appointment as full Professor was made the following year.

Dr. Wangensteen has been active in numerous scientific societies and has received many awards for his contributions to clinical surgery and surgical research. Among the awards which he has received are the Samuel D. Gross prize of the Philadelphia Academy of Surgery and a LL.D. degree from the University of Buffalo in 1946. In addition to making many contributions to medical journals, he serves as co-editor of the scientific journal, *Surgery*.