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3,486,981

SUBSTANCES RELATING TO TESTING OF BLOOD-COAGULATION

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No Drawing. Filed Mar. 15, 1965, Ser. No. 439,941
Int. Cl. C12k 1/10; G01n 33/16

U.S. Cl. 195—99

13 Claims

ABSTRACT OF THE DISCLOSURE

A partial thromboplastin time test reagent and a multi-factor coagulation reagent, having a chemical activator, the reagents providing good stability, high sensitivity, and a more reproducible and accurate evaluation of a patient's coagulability state. Hageman factor is maximally activated in a constant manner, yielding precision and reliability of test results. The multi-factor coagulation reagent is sensitive to both the intrinsic and extrinsic coagulation systems, advantageously permitting both systems to be tested in a single diagnostic procedure.

This invention relates to a new discovery in medical diagnosis, specifically in the field of laboratory diagnosis of coagulation disorders, and more particularly, in substances and methods useful therein and relating thereto.

CURRENT BLOOD COAGULATION THEORY—INTRINSIC TYPE

For purpose of clarification and background, a brief description of the current coagulation theory is presented introductorily, as now set forth.

If blood from a person's vein is placed in a glass test-tube and observed, it is seen, after a certain length of time, that the blood changes from fluid to a gel. This is the process of coagulation of the so-called "intrinsic type." It is termed "intrinsic" for the reason that all of the coagulation factors involved in the reaction are of intravascular origin. As explained below, the process occurs, basically, in a three-step reaction: (1) formation of blood thromboplastin, (2) conversion of prothrombin, (3) production of gel (clot).

CURRENT THEORY—FORMATION OF BLOOD THROMBOPLASTIN

Platelets (blood cells of a certain type) release one or more "platelet factors" as a result of physical contact with the surface of the glass test tube. The platelet factor reacts with several coagulation factors present in normal blood, comprising anti-hemophilia globulin, plasma thromboplastic antecedent, plasma thromboplastic component, Stuart-Prower factor, Hageman factor (which is also activated by the glass surface), calcium ions, and perhaps other unknown factors to produce "blood thromboplastin."

Diagrammatically, this might be shown as follows:

Platelet factor, plus
Plasma thromboplastic antecedent, plus
Plasma thromboplastic component, plus
Stuart-Prower factor, plus
Hageman factor (surface activation), plus
Antihemophilia globulin, plus
Calcium ions

"Blood thromboplastin"

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CURRENT THEORY—CONVERSION OF PROTHROMBIN

"Blood thromboplastin," in the presence of other factors, comprising Stuart-Prower factor, proaccelerin, and calcium ions, converts prothrombin to thrombin.

Prothrombin
↓
"Blood thromboplastin", plus
Stuart-Prower factor, plus
Proaccelerin, plus
Calcium ions
↓
Thrombin

CURRENT THEORY—PRODUCTION OF GEL (CLOT)

Thrombin reacts with the soluble plasma protein, fibrinogen, and converts it to the insoluble fibrin in the form of a gel.

Fibrinogen
↓
Thrombin
↓
Fibrin

INTRINSIC COAGULATION—TIME AND OTHE FACTORS

This intrinsic system is a relatively slow reaction. The time required for the reaction to go to completion depends on several conditions, such as temperature, concentration of coagulation factors, and degree of physical surface activation.

CURRENT BLOOD COAGULATION THEORY—EXTRINSIC TYPE

Besides the "intrinsic system" just discussed, there is a second coagulation system, known as "the extrinsic system." The term "extrinsic," is used because one of the factors involved is extra-vascular in origin. This other factor is known as "tissue thromboplastin," and is present in nearly all tissues of the body. The "extrinsic system" reaction, basically, is a two-step reaction. The production of thromboplastin in this extrinsic system is unnecessary, since it exists in a pre-formed state as tissue thromboplastin.

CURRENT THEORY—CONVERSION OF PROTHROMBIN

Tissue thromboplastin in the presence of Stuart-Prower factor, proaccelerin, proconvertin, and calcium ions, converts prothrombin to thrombin.

Prothrombin
↓
Tissue thromboplastin, plus
Proconvertin, plus
Proaccelerin, plus
Stuart-Prower factor, plus
Calcium ions
↓
Thrombin

CURRENT THEORY—PRODUCTION OF GEL (CLOT)

Thrombin reacts with fibrinogen, and converts it to the insoluble fibrin.

Fibrinogen
↓
Thrombin
↓
Fibrin

EXTRINSIC COAGULATION—TIME AND OTHER FACTORS, AND COMPARISON TO INTRINSIC SYSTEM

The "extrinsic system" of coagulation is relatively rapid (10–15 seconds), in contrast to the "intrinsic system" of coagulation (4–5 minutes). There are some other basic differences. Blood thromboplastin does not require proconvertin for its activity in conversion of prothrombin to thrombin, whereas, tissue thromboplastin does. Surface activation has a dramatic effect on the "intrinsic system," but only a minor one on the "extrinsic system."

CHEMICAL ACTIVATION OF HAGEMAN FACTOR

The new discovery mentioned in the first paragraph involves, as one thing, the chemical activation of Hageman factor. This process, to be described below, makes it possible to improve certain laboratory tests for the assessing of the activity of certain coagulation factors of blood. This chemical activation of Hageman factor includes the following procedures and components:

- (1) Use of ellagic acid, or one of its related compounds or derivatives of these compounds, in the partial thromboplastin time test;
- (2) Process for preparing a stable solution of the chemical activator;
- (3) Preparation of a reagent consisting of a platelet substitute and the chemical activator; and its use as a blood test for testing coagulation factors; and
- (4) Preparation of a reagent consisting of a platelet substitute, chemical activator, and tissue thromboplastin; and its use as a blood test for testing coagulation factors.

THE USE OF ELLAGIC ACID, OR ONE OF ITS RELATED COMPOUNDS OR ONE OF THE DERIVATIVES OF THESE COMPOUNDS, IN THE PARTIAL THROMBOPLASTIN TIME TEST

It has recently been reported that ellagic acid, and other di-hydroxy substituted diphenyl compounds, maximally activate Hageman factor. Although other related compounds also function, ellagic acid seems to be the most effective. Ellagic acid maximally activates Hageman factor in a concentration of 10^{-8} to 10^{-4} molar. Furthermore, it apparently does not influence any other portion of the coagulation process, functioning neither as an inhibitor nor as an accelerator.

PTT TEST—COAGULATION DEFICIENCIES

The partial thromboplastin time test (PTT) is a laboratory test used to screen for coagulation disorders.

It detects deficiencies in the following coagulation factors:

- (1) Hageman factor;
- (2) Stuart-Prower factor;
- (3) Plasma thromboplastic antecedent;
- (4) Plasma thromboplastic component;
- (5) Anti-hemophilia globulin;
- (6) Prothrombin;
- (7) Proaccelerin; and
- (8) Fibrinogen.

It does not detect deficiencies of the following coagulation factors:

- (1) Platelets;
- (2) Proconvertin;
- (3) Calcium; or
- (4) Fibrin stabilizing factor.

The PTT test will also detect the presence of circulating anti-coagulants, such as anti-thromboplastin and anti-thrombin,

PTT TEST—GENERAL NATURE

The PTT test is an in vitro simulation of the "intrinsic system" of coagulation. The basic reagent is a platelet substitute in optimum concentration. A platelet substitute is used because of the technical difficulty of preparing a standard platelet suspension.

PTT TEST—ORIGINAL VERSION

The PTT test of Langdell, Wagner, and Brinhouse (J. Lab. and Clin. Med., vol. 41, page 637, 1953) is performed in the following manner: The platelet substitute is mixed with oxalated or citrated plasma. To this is added sufficient calcium in solution to neutralize the anti-coagulant and to provide an optimal concentration of calcium ions for the coagulation reaction. The test is performed at 37° C. The time required for the formation of a clot is determined. In case of an abnormality, the time is prolonged. The normal range for the original procedure is 40 to 100 seconds.

PTT TEST—DEFECTS OR DIFFICULTIES OF ORIGINAL VERSION

The main defect of the above procedure lies in the fact that glass is an unreliable physical activator of Hageman factor. It neither activates maximally nor consistently. Even the manner in which glass is washed influences the degree to which it will activate Hageman factor. Reproducibility is thus poor.

PTT TESTS—SUBSEQUENT MODIFICATIONS

Attempts have been made to remedy this unreliability by utilizing insoluble activators, such as kaolin, celite, bentonite, and others. These activators do activate maximally, but the concentration of the activator is critical. If there is not enough activator, maximal activation does not occur. If too much activator is used, absorption of Hageman factor, and perhaps other factors, occurs, resulting in loss of activity.

Furthermore, the time of maximum activity is critical and of short duration, so that the test must be performed at an accurately timed interval of activation.

The normal range for the procedure utilizing kaolin or celite is 35 to 45 seconds. This version of the PTT test increased the sensitivity of the test, mainly due to the increased reproducibility of results. However, there are still disadvantages, as mentioned above, particularly with respect to time of activation and concentration of the activator.

PTT TEST—CHEMICAL ACTIVATION

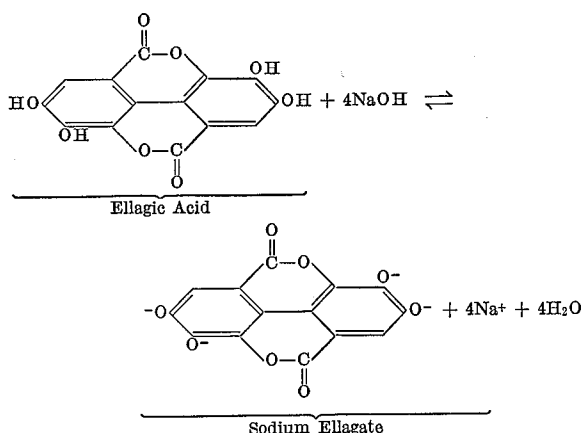
These disadvantages have been overcome according to the present invention, by the introduction of a chemical activator of Hageman factor. Using a solution of ellagic acid, activation was rapid, less than one minute. Also, the activation was stable. The plasma remains fully activated for as long as 60 minutes. Ellagic acid was used with three commercial partial thromboplastins in separate tests: Cephaloplastin (Dade), Platelin (Warner-Chilcott), and Thrombofax (Ortho). Equivalent results were obtained with each. The normal range was 32 to 38 seconds. The standard deviation was 0.5 second, and represents superior precision. The short normal range represents increased sensitivity to coagulation defects.

PREPARATION OF STABLE SOLUTION OF CHEMICAL ACTIVATOR

Ellagic acid was reagent prepared according to Ratnoff and Crum (J. of Lab. and Clin. Med., vol. 63, pages 359–377, 1964), but it was not stable, and it precipitated after standing a few days. This was remedied according to the present invention by converting the ellagic acid

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to its sodium salt, by adding sodium hydroxide, this reaction, it is believed, being as follows:



This was followed by filtration. The resulting solution retained the full activity of the ellagic acid.

COMBINING PARTIAL THROMBOPLASTIN AND A SALT OF ELLAGIC ACID IN A SINGLE REAGENT

Utilizing the previous principles, the sodium salt of ellagic acid and partial thromboplastin may be prepared in a single reagent; and each component is found to have no deleterious effect on the other over an extended time. This reagent functions identically as do the components when used separately.

COMBINING PARTIAL THROMBOPLASTIN, OF A SALT ELLAGIC ACID, AND TISSUE THROMBOPLASTIN IN A SINGLE REAGENT

In order to make a reagent sensitive to both the "intrinsic" and "extrinsic" systems, tissue thromboplastin was incorporated in the above reagent, at a concentration set forth and discussed hereinbelow.

PREPARATION AND TEST DETAILS

(I) Preparation of stable ellagic acid solution (sodium salt)

(1) 0.034 gm. (10^{-4} mole) ellagic acid is weighed, and transferred to a 50 ml. beaker.

(2) 5 ml. 0.1 M sodium hydroxide are added to dissolve the ellagic acid. (Sufficient sodium hydroxide is added in order to convert the ellagic acid to sodium ellagate with a slight excess of sodium hydroxide remaining.)

(3) Dilute to 1000 ml. with distilled water in a 1000 ml. volumetric flask, stopper, and mix well.

(4) Filter through a 5.0μ pore millipore filter.

(II) Preparation of stable ellagic acid solution (potassium salt)

(1) 0.034 gm. (10^{-4} mole) ellagic acid is weighed on analytical balance and transferred to a 50 ml. beaker.

(2) 5 ml. 0.1 M potassium hydroxide are added to dissolve the ellagic acid. (Sufficient potassium hydroxide is added to convert the ellagic acid to potassium ellagate with a slight excess of potassium hydroxide remaining.)

(3) Dilute to 1000 ml. with distilled water in a 1000 ml. volumetric flask, stopper, mix well.

(4) Filter through a 5.0μ pore millipore filter.

(III) Preparation of stable ellagic acid solution (ammonium salt)

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(1) 0.034 gm. (10^{-4} mole) ellagic acid is weighed on analytical balance and transferred to a 50 ml. beaker.

(2) 5 ml. 0.1 M ammonium hydroxide are added to dissolve the ellagic acid. (Sufficient ammonium hydroxide is added to convert the ellagic acid to ammonium ellagate with a slight excess of ammonium hydroxide remaining.)

(3) Dilute to 1000 ml. with distilled water in a 1000 ml. volumetric flask, stopper, and mix well.

(4) Filter through a 5.0μ pore millipore filter.

(IV) Comparison of ellagic acid and its salts in the partial thromboplastin time test

Dilutions were made with distilled water of the sodium ellagate, potassium ellagate, and ammonium ellagate solutions, so that the following concentrations were obtained: 10^{-5} , 10^{-6} , 10^{-7} , 10^{-8} molar.

The above dilutions of each of the ellagate solutions (as well as ellagic acid) were tested in the partial thromboplastin test using pooled normal plasma (5 blood donors), a patient under dicoumarol therapy (acquired deficiencies of Prothrombin, proconvertin, Stuart-Prower factor), one patient with hemophilia (deficiency of anti-hemophilia globulin), and one patient with Christmas disease (deficiency of plasma thromboplastic component).

The results are listed in Charts I, II, III, and IV.

CHART I.—PARTIAL THROMBOPLASTIN TIME USING ELLAGIC ACID AT VARIOUS CONCENTRATIONS IN COMBINATION WITH PLATELET SUBSTITUTE PREPARED ACCORDING TO THE PROCEDURE OF BELL AND ALTON

[Bell, W. N., and Alton, H. G.: Brain extract as a substitute for platelet suspension in the thromboplastin generation test. Nature, London, 174: 880-881, 1954]

	Concentration of Ellagic Acid (sec.)				
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Pooled Normal Plasma.....	34	33	34	34	32
Hemophilic Plasma.....	128	130	128	129	130
Christmas Disease Plasma.....	114	115	115	116	115
Dicoumarolized Plasma.....	118	118	120	120	118

CHART II.—PARTIAL THROMBOPLASTIN TIME USING SODIUM ELLAGATE AT VARIOUS CONCENTRATIONS IN COMBINATION WITH PLATELET SUBSTITUTE PREPARED ACCORDING TO THE PROCEDURE OF BELL AND ALTON

	Concentration of Sodium Ellagate (sec.)				
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Pooled Normal Plasma.....	36	33	36	38	37
Hemophilic Plasma.....	125	130	126	126	125
Christmas Disease Plasma.....	110	114	114	112	110
Dicoumarolized Plasma.....	116	118	118	120	118

CHART III.—PARTIAL THROMBOPLASTIN TIME USING POTASSIUM ELLAGATE AT VARIOUS CONCENTRATIONS IN COMBINATION WITH PLATELET SUBSTITUTE PREPARED ACCORDING TO THE PROCEDURE OF BELL AND ALTON

	Concentration of Potassium Ellagate (sec.)				
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Pooled Normal Plasma.....	33	36	33	32	33
Hemophilic Plasma.....	132	131	131	128	127
Christmas Disease Plasma.....	116	118	114	116	118
Dicoumarolized Plasma.....	120	119	116	118	120

CHART IV.—PARTIAL THROMBOPLASTIN TIME USING AMMONIUM ELLAGATE AT VARIOUS DILUTIONS IN COMBINATION WITH PLATELET SUBSTITUTE PREPARED ACCORDING TO THE PROCEDURE OF BELL AND ALTON

	Concentration of Ammonium Ellagate (sec.)				
	10^{-4} M	10^{-5} M	10^{-6} M	10^{-7} M	10^{-8} M
Pooled Normal Plasma.....	37	38	35	37	34
Hemophilic Plasma.....	130	134	131	130	129
Christmas Disease Plasma.....	115	118	116	118	117
Dicoumarolized Plasma.....	120	118	118	117	118

(V) Procedures used for tests

(1) A commercial partial thromboplastin reagent, Cephaloplastin (Dade) was reconstituted with the various dilutions of the ellagate solutions.

(2) The blood specimens used were collected in 0.1 volume 0.1 M sodium citrate. The blood was centrifuged at 1500 r.p.m. for 10 minutes, and the supernatant plasma removed. All of the plasmas were tested within thirty minutes of the collection.

(3) 0.1 ml. reconstituted Cephaloplastin was placed in two 10 x 75 mm. glass test tubes.

(4) To each test tube was added 0.1 ml. plasma to be tested, and the test tubes were placed in a 37° C. water bath for three minutes in order for the mixture to come to 37° C.

(5) 0.1 ml. 0.02 M calcium chloride was blown into the mixture, and simultaneously a stop watch was started. After thirty seconds, the tube was gently tilted, and the time required for formation of clot was noted.

(VI) Results

It may be seen from Charts I through IV, above, that equivalent results were obtained with ellagic acid and the following monovalent salts: sodium, potassium and ammonium. Furthermore, no obstacle is foreseen which would prevent the use of other monovalent cationic salts of ellagic acid in the partial thromboplastin time test. Moreover, the results were equivalent with normal as well as abnormal plasmas.

(VII) Various hydroxy-substituted aromatic compounds in the partial thrombolastin time test

10⁻⁴ molar solutions of the sodium salts of the following hydroxy-substituted compounds: 3,4-dihydroxyphenylethylamine (dopamine), 1,2 - dihydroxy - anthraquinone (alizarin), 2,7,8,9 - tetrahydroxytropone (purpurogallin) and 4,4',5,5',6,6'-hexahydroxydiphenic acid, 2,6:2,6''-dilactone (ellagic acid), were prepared in the same manner as in section I, and used in the same manner as in section V, above.

(VIII) Results

Equivalent results were obtained with all solutions, showing that these hydroxy-substituted compounds are interchangeable in the partial thromboplastin time test. See Chart V, below.

CHART V.—PARTIAL THROMBOPLASTIN TIME USING VARIOUS HYDROXY-SUBSTITUTED AROMATIC COMPOUNDS IN COMBINATION WITH PLATELET SUBSTITUTE PREPARED ACCORDING TO PROCEDURE OF BELL AND ALTON. THE CHEMICAL ACTIVATORS WERE USED AT A CONCENTRATION OF 10⁻⁴ M

	Seconds			
	Sodium* Dopamine	Sodium Alizarin	Sodium Purpuro gallin	Sodium Ellagate
Pooled Normal Plasma...	36	35	36	34
Hemophilic Plasma...	133	128	131	128
Christmas Disease Plasma...	117	119	118	117
Dicoumarolized Plasma...	121	116	119	116

*Aged for 48 hours. Aging necessary for maximum activity.

(IX) Comparison of various platelet substitutes combined with sodium ellagate in the partial thromboplastin time test

CHART VI.—PARTIAL THROMBOPLASTIN TIME USING SODIUM ELLAGATE (10⁻⁴ M) AND VARIOUS PLATELET SUBSTITUTES

	Type of Platelet Substitute (sec.)		
	Cephaloplastin (Dade)	Thrombofax (Ortho)	Platelin (Warner-Chilcott)
Pooled Normal Plasma.....	34	37	40
Hemophilic Plasma.....	124	128	125
Christmas Disease Plasma.....	114	112	114
Dicoumarolized Plasma.....	118	120	121

(X) Effect of various temperatures on the degree of activation in the partial thromboplastin time test, using platelet substitute and sodium ellagate

CHART VII.—EFFECT OF TEMPERATURE ON DEGREE OF ACTIVATION IN THE PARTIAL THROMBOPLASTIN TIME TEST USING SODIUM ELLAGATE (10⁻⁴ M) AND CEPHALOPLASTIN (DADE). ACTIVATION TIME, FIVE MINUTES

	Temperature		
	4° C. (sec.)	25° C. (sec.)	37° C. (sec.)
Pooled Normal Plasma.....	34	34	35
Hemophilic Plasma.....	129	132	130
Christmas Disease Plasma.....	112	112	114
Dicoumarolized Plasma.....	116	115	118

(XI) Stability of activation in the partial thromboplastin time test, using platelet substitute and sodium ellagate

CHART VIII.—STABILITY OF ACTIVATION IN THE PARTIAL THROMBOPLASTIN TIME USING SODIUM ELLAGATE (10⁻⁴ M) AND CEPHALOPLASTIN (DADE). ACTIVATION TEMPERATURE, 37° C.

	Activation time in minutes				
	1	5	15	30	60
Pooled Normal Plasma (sec.).....	34	34	34	35	34
Hemophilic Plasma (sec.).....	126	128	125	125	126
Christmas Disease Plasma (sec.).....	116	116	117	116	116
Dicoumarolized Plasma (sec.).....	110	112	110	113	110

(XII) Multi-factor coagulation reagent

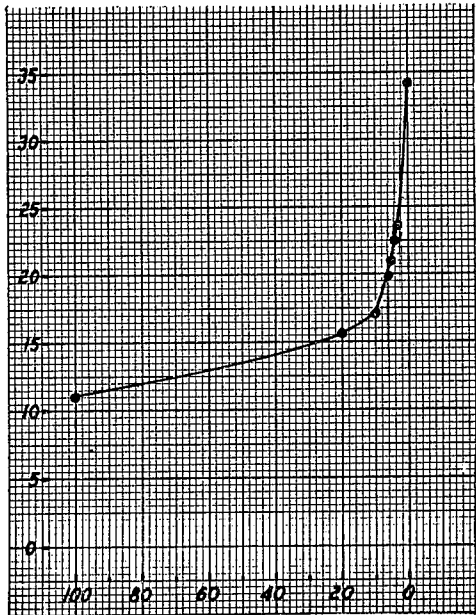
(A) Partial thromboplastin time reagent utilizing platelet substitute and chemical activator may be made sensitive to both the "intrinsic" and "extrinsic" coagulation systems by incorporation of the appropriate amount of tissue thromboplastin into the partial thromboplastin reagent.

This sensitivity to all factors (except platelets) of both "intrinsic" and "extrinsic" coagulation systems is a sensitivity far greater than merely a sensitivity to plasma thromboplastic component, Stewart-Prower factor, proconvertin, and prothrombin, as in the Thrombotest of Owren (Saertrykk av Farmakoterapi nr. 4, vol. XV, 1959. Utgiver: Nyegaard & Co. A/S, Oslo).

The amount of tissue thromboplastin used is that quantity that gives clotting times intermediate between the one-stage Prothrombin Time (Quick, A.J.: The Hemorrhagic Diseases and the Physiology of Hemostasis, p. 312. Charles C. Thomas, Springfield, Ill., 1942) and the Partial thromboplastin time, a desired mixture being in the range of 1% to 16% as shown in Chart IX.

CHART IX.—EFFECT OF VARIOUS CONCENTRATIONS OF TISSUE THROMBOPLASTIN WITH OPTIMUM CONCENTRATION OF SODIUM ELLGATE (10^{-4} M) AND CEPHALOPLASTIN (DADE) IN A MODIFIED PARTIAL THROMBOPLASTIN TIME TEST. NORMAL PLASMA USED

MODIFIED PARTIAL THROMBOPLASTIN TIME IN SECONDS



PERCENT TISSUE THROMBOPLASTIN

(B) Each lot of the reagent must be standardized by preparation of a dilution curve such as shown in Chart IX. The reagent is stabilized either by incorporating 3% phenol or by freeze drying.

Accordingly, it is seen that the concepts of the present invention provide reagents which do the following:

(1) Makes available a more sensitive partial thromboplastin time reagent.

(2) Makes available a PTT reagent in which Hageman factor is maximally activated and this activation remains constant throughout the test procedure.

(3) Makes available a PTT reagent which is stable with with storage.

(4) Makes available a multi-factor reagent sensitive to both the intrinsic and extrinsic coagulation system.

(5) Makes available a multi-factor reagent stable with storage.

What is claimed is:

1. A partial thromboplastin time test reagent comprising a platelet substitute, and ellagic acid as the chemical activator.

2. A partial thromboplastin time test reagent comprising a platelet substitute, and sodium ellagate as the chemical activator.

3. A partial thromboplastin time test reagent comprising a platelet substitute, and potassium ellagate as the chemical activator.

4. A partial thromboplastin time test reagent comprising a platelet substitute, and ammonium ellagate as the chemical activator.

5. A partial thromboplastin time test reagent comprising a platelet substitute, and a monovalent salt of ellagic acid as the chemical activator.

6. A partial thromboplastin time test reagent comprising a platelet substitute, and an hydroxy-substituted aromatic compound as the chemical activator.

7. A partial thromboplastin time test reagent comprising a platelet substitute, and a salt of an hydroxy-substituted aromatic compound as the chemical activator.

8. A partial thromboplastin time test reagent comprising a platelet substitute, and a monovalent salt of an hydroxy-substituted aromatic compound as the chemical activator.

CO-ORDINATES

X	Y
100	12
80	15.6
60	17
40	19.8
20	21
10	22.5
5	27
3	34.2

9. A multi-factor coagulation reagent comprising platelet substitute, tissue thromboplastin, and sodium ellagate as the chemical activator.

10. A multi-factor coagulation reagent comprising platelet substitute, tissue thromboplastin, and potassium ellagate as the chemical activator.

11. A multi-factor coagulation reagent comprising platelet substitute, tissue thromboplastin, and ammonium ellagate as the chemical activator.

12. A multi-factor coagulation reagent comprising platelet substitute, tissue thromboplastin, and a monovalent salt of ellagic acid as the chemical activator.

13. A multi-factor coagulation reagent comprising platelet substitute, tissue thromboplastin, and a monovalent salt of an hydroxy-substituted aromatic compound.

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MORRIS O. WOLK, Primary Examiner

R. M. REESE, Assistant Examiner

U.S. Cl. X.R.

23—230; 195—103.5; 252—408; 260—343.2; 324—2, 95

UNITED STATES PATENT OFFICE
CERTIFICATE OF CORRECTION

Patent No. 3,486,981

December 30, 1969

Roy E. Speck

It is certified that error appears in the above identified patent and that said Letters Patent are hereby corrected as shown below:

Column 2, line 25, "OTHE" should read -- OTHER --. Column 4, line 71, "was reagent" should read -- reagent was --. Column 5, lines 40 and 41, "OF A SALT" should read -- A SALT OF --. Column 6, line 5, "acide" should read -- acid --; line 30, "PRL-" should read -- PRE- --; line 42, "ELLAGAGE" should read -- ELLAGATE --.

Signed and sealed this 10th day of November 1970.

(SEAL)

Attest:

Edward M. Fletcher, Jr.

Attesting Officer

WILLIAM E. SCHUYLER, JR.

Commissioner of Patents