Check for updates

Clot Firmness

By Murray Weiner and Louis G. Weisberg

DEFECTS OF CLOTTING are generally studied by estimating the time necessary for visible clot formation under a variety of conditions. It is generally recognized that some clot abnormalities are not adequately reflected in clotting time tests. Observations of retraction and lysis of clots represent attempts to study qualities of clots other than those reflected in the time necessary for clotting to take place. Methods for quantifying other qualities of a clot are notoriously unsatisfactory.

It is generally recognized that a fresh gelatinous clot tends to become firmer with retraction, and that under various conditions clots fail to develop normal firmness. This paper deals with firmness of clots as measured by the coagulograph ("Thrombelastograph" of Hartert[1]) an instrument previously employed by us for studying both quantitative and qualitative variations in fibrinolytic activity.[2] Observations concerning some of the factors which influence clot firmness as measured by the coagulograph will be presented.

Methods and Materials

Firmness of clots (i.e., resistance to distortion) was estimated by the Hartert instrument[1] which records continuously the degree to which a standardized to-and-fro spin of a cup is transmitted to a suspended plunger which is in contact with the cup only via a 1 mm. thickness of intervening clot (fig. 1). The instrument records a straight line on photographic paper moving at 2 mm. per minute when the clot is not yet formed or is too easily distended to transmit cup motion to the plunger. With increasing firmness, the plunger begins to follow the spin of the cup, and converts the single line record into 2 diverging lines. When firmness becomes constant, the recorded lines become parallel. The maximum amplitude (m.a.) of the divergent lines in millimeters is considered to be a quantitative estimation of clot firmness.*

All tests were performed with a final reaction mixture of 0.35 ml. Calcium activated mixtures included the addition of 0.01 ml. of 0.5 Molar calcium chloride (i.e., 0.015 Molar added calcium chloride in the final reaction mixture) unless otherwise noted.

Citrated plasma was obtained by adding 4.5 ml. fresh blood to 0.5 ml. 3.8% sodium citrate in glass. The specimens were spun at 500 RPM for 5 minutes and plasma separated. Platelet preparations were made by differential centrifugation using siliconized equipment.

Data

Figure 2A compares a typical coagulograph of normal citrated plasma activated by calcium with the patterns obtained by activating directly with thrombin.† It can be seen that the "thrombin" clot is considerably less firm than that formed by recalcification. However, the recalcified specimen clotted and developed its

From the Research Service, Third (New York University) Medical Division, Goldwater Memorial Hospital, New York, N. Y.

Submitted March 18, 1957; accepted for publication July 16, 1957.

* The discussion in a previous report (2) presents our reason for believing that this instrument does not measure the elasticity of a clot, as claimed in the inventor's original description, but rather the firmness of a clot.

† Thrombin was obtained as a dry preparation (Ortho) through the courtesy of Dr.

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023

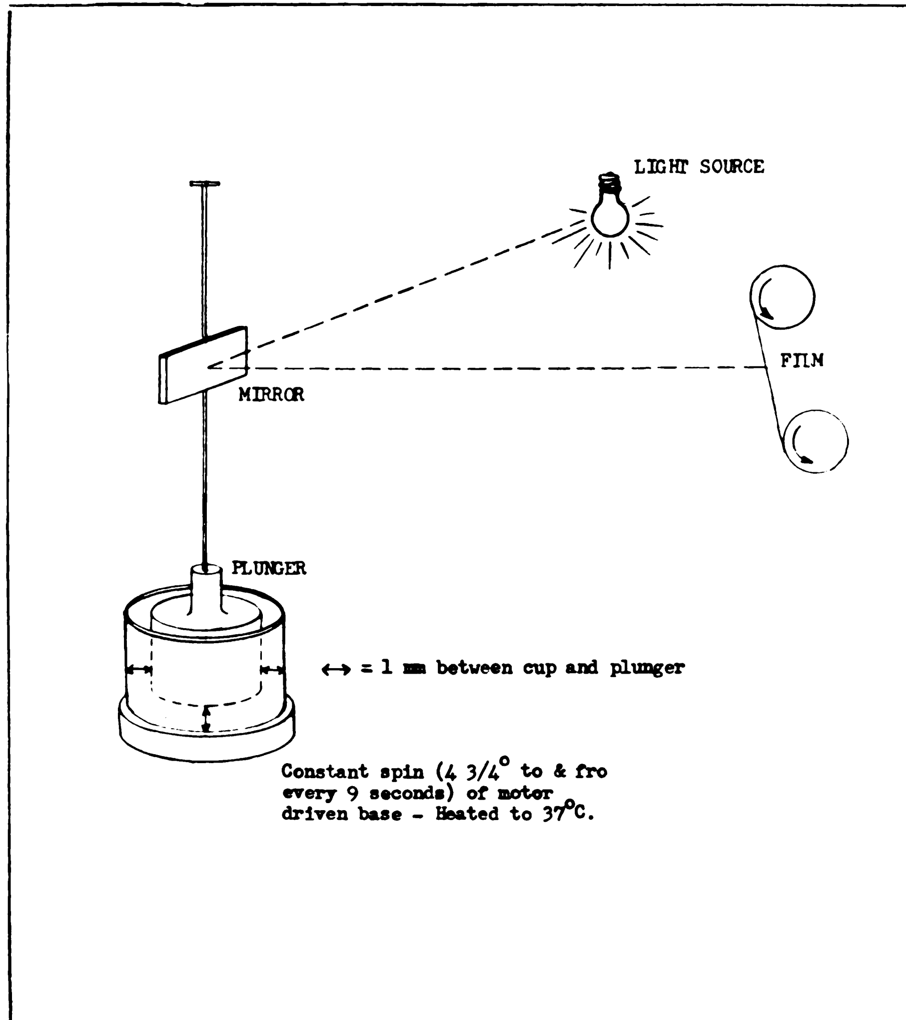


FIG. 1.—Schematic drawing of the "thrombelastograph."

full firmness much later than the thrombin-activated aliquot. If clotting of citrated plasma is initiated by the simultaneous addition of both thrombin and $CaCl_2$, the clot forms rapidly and develops full firmness rapidly (fig. 2A). This is even more dramatically noted with hemophilic plasma, which usually takes

Phillip Levine. The material was dissolved in saline to make fresh solutions of appropriate concentrations.

Fibrinogen was obtained as a citrate-free dry preparation (Chilcott) through the courtesy of Dr. George Phillips. A preparation of bovine fibrinogen containing 45% citrate (Armour) was also studied.

Bacterial coagulase was prepared and kindly supplied by Dr. Morris Tager of Emory University. Preliminary test tube clotting time determinations with citrated plasma indicated that 1.0 mg. of this coagulase preparation was equivalent in clotting time activity to approximately 5 units of thrombin.

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023

hours to develop its full firmness (fig. 2C). High speed centrifuged ("low platelet") hemophilic plasma shows the same effect, except that its full firmness is considerably less than that of the "normal platelet" plasma.

The addition of thrombin to solutions of "purified" fibrinogen† results in firmer clots than those obtained with citrated plasma specimens of equivalent fibrinogen concentration (compare fig. 3B with fig. 2A). With fibrinogen solutions, there is a clear influence of thrombin concentration on clot firmness (fig. 3B and 4C) not seen in plasma specimens activated by thrombin (fig. 2B).

There is a considerable influence of citrate concentration of plasma specimens on clot firmness (fig. 4A). When citrate concentration was kept constant, and calcium concentration varied, it was found that greater clot firmness was achieved with higher calcium concentrations, although these higher calcium concentrations did not significantly influence the rate of clot formation (fig. 4B). However, a preparation of bovine fibrinogen (Armour) containing about 45% citrate, was converted into a moderately firm clot by the addition of thrombin and without adding calcium (fig. 4C).

The concentration of fibrinogen affects the firmness of plasma clots (fig. 3A) as well as "purified" clotting systems (fig. 3B).

Platelets have been shown to have a marked influence on clot firmness.[2] However, this influence of platelets, clearly detectable with citrated plasma activated by recalcification, is not as evident when citrated plasma is clotted by thrombin without the addition of calcium (fig. 4D, 4E).

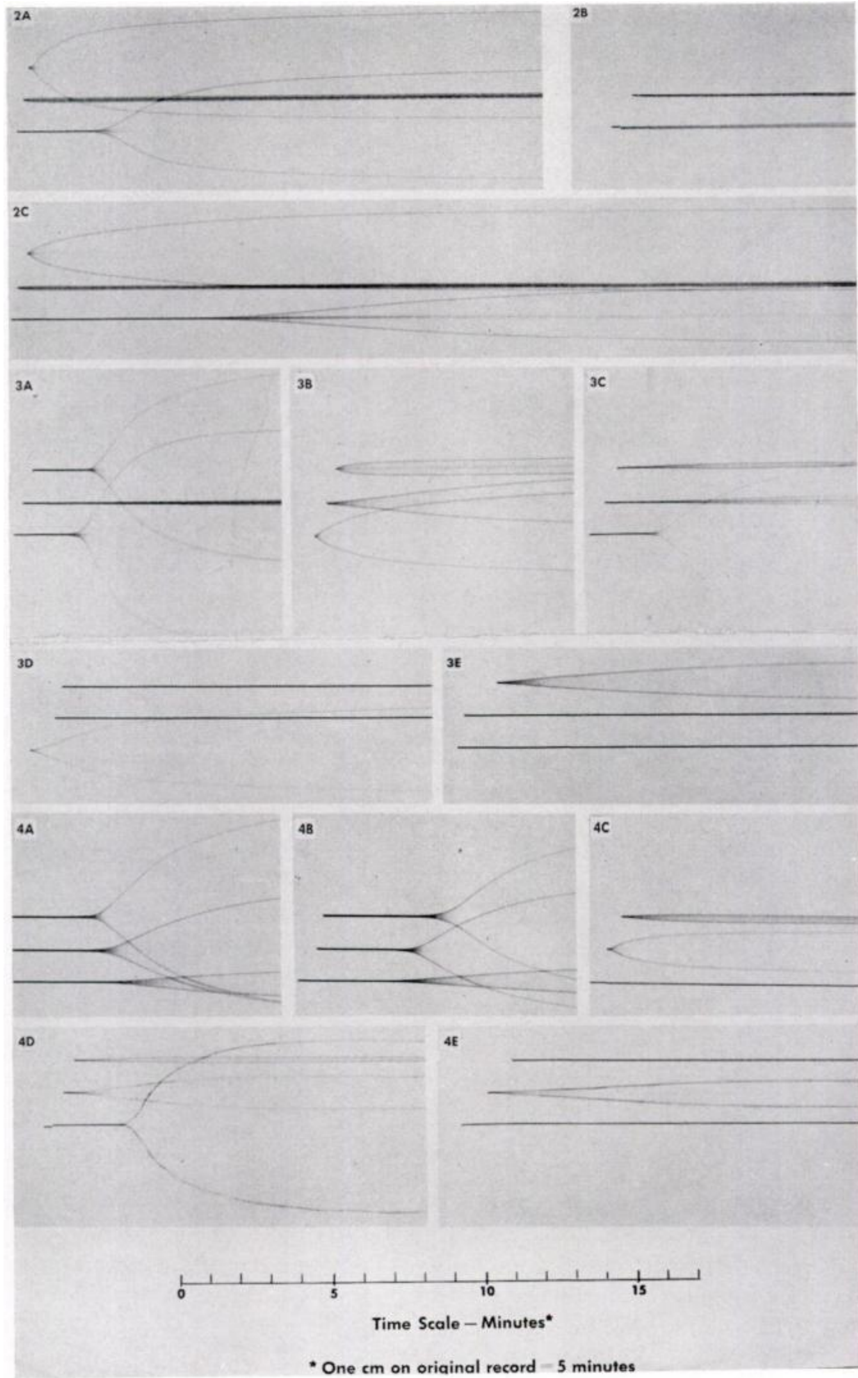
The patterns of clot firmness formed by the addition of bacterial coagulase† was found to differ from that of both thrombin-activated and calcium activated clots. Like thrombin, coagulase will clot both citrated plasma and "purified" fibrinogen. However, unlike thrombin, coagulase yields a firmer clot with activated plasma than with fibrinogen (fig. 3C, D, E). The relative influence of calcium, thrombin and coagulase on clot firmness is summarized in the typical example presented in table 1.

It was repeatedly noted that clot firmness becomes diminished on storage of citrated plasma. Clot firmness was reduced more rapidly by 24 hours of rapid deep freeze storage than a similar period at 6 C. (table 2). This reduced firmness was not accompanied by a slower rate of clot formation. Aliquots stored for 2 weeks no longer showed this difference between deep frozen and refrigerator stored specimens (table 2).

Discussion

The data clearly indicate that clot firmness as measured by the coagulograph maximum amplitude bears little relation to the speed of clot formation. Judging from platelet deficiency states, clot firmness is apparently more closely related to clot retraction than any other commonly used clotting measurement. Clot firmness will vary with fibrinogen concentration, calcium concentration and platelet alterations. Increasing clot firmness can be demonstrated to result from increasing thrombin concentration in purified fibrinogen systems, but is much less evident in whole plasma systems. The fact that plasma clotted by thrombin is less firm than an equivalent solution of fibrinogen clotted by thrombin suggests

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023



FIGS. 2A–4E

FIG. 2.—A. Normal citrated plasma activated by:
Upper graph: Calcium chloride and thrombin (6 units/ml. plasma)
Middle graph: Thrombin (6 units/ml. plasma)
Lower graph: Calcium chloride

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023

that: (a) the fibrinogen had been "activated" in the process of preparation, or (b) the fibrinogen preparation contains other factor(s) which enhance firmness, or (c) plasma contains factor(s) which inhibit firmness.

It is possible that the hypothetical "firmness factor" is related to platelets. It remains to be determined which of the numerous platelet components may be involved. Previous studies[3] have ruled out platelet serotonin as a factor responsi-

---

B. "Low platelet" citrated plasma (20,000 platelets per cu.mm.) activated by:
Upper graph: 6 units thrombin per ml. plasma
Lower graph: 30 units thrombin per ml. plasma
C. Hemophilic citrated plasma activated by:
Upper graph: Calcium chloride and thrombin (6 units per ml. plasma)
Middle graph: Thrombin (6 units per ml. plasma)
Lower graph: Calcium chloride
FIG. 3.—A. Citrated plasma activated with calcium chloride:
Upper curve: Same as lower, with 1.5 units streptokinase per ml. plasma added
Middle curve: Patient with hypofibrinogenemia (56 mgm. fibrinogen per 100 ml. plasma)
Lower curve: Patient with thrombophlebitis
B. "Purified" fibrinogen (Chilcott) plus thrombin
Upper curve: 3 mg./ml. fibrinogen plus 30 units/ml. thrombin
Middle curve: 6 mg./ml. fibrinogen plus 6 units/ml. thrombin
Lower curve: 6 mg./ml. fibrinogen plus 30 units/ml. thrombin
C. Normal citrated plasma activated by:
Upper graph: 0.3 units thrombin per ml. plasma
Middle graph: 72 μg. coagulase per ml. plasma
Lower graph: Calcium chloride
D. "Purified" fibrinogen activated by:
Upper graph: 1.0 mg./ml. coagulase (3 mg. fibrinogen/ml.)
Middle graph: 1.0 mg./ml. coagulase (6 mg. fibrinogen/ml.)
Lower graph: 6.0 units/ml. thrombin (6 mg. fibrinogen/ml.)
E. "Purified" fibrinogen (6 mg./ml.) activated by:
Upper graph: Coagulase (0.5 mg./ml.) plus thrombin (3 units/ml.)
Middle graph: Coagulase (0.5 mg./ml.) plus calcium chloride
Lower graph: Coagulase (0.5 mg./ml.)
FIG. 4.—A. Normal plasma (Calcium chloride activated) with varying concentration of sodium citrate in original blood specimen.
Upper graph: 0.25% sodium citrate (0.0066 Molar)
Middle graph: 0.38% sodium citrate (0.010 Molar)
Lower graph: 0.54% sodium citrate (0.014 Molar)
B. Normal citrated plasma activated by varying concentrations of calcium chloride.
Upper graph: 0.030 Molar calcium chloride
Middle graph: 0.015 Molar calcium chloride
Lower graph: 0.0075 Molar calcium chloride.
C. Citrate-contaminated fibrinogen (Armour) 20 mg./ml. activated by varying thrombin concentrations.
Upper graph: 3 μ thrombin per ml.
Middle graph: 18 μ thrombin per ml.
Lower graph: No thrombin. Calcium chloride added
FIG. 4.—D. Activation of "high platelet" plasma by:
Upper graph: thrombin (0.3 μ/ml.)
Middle graph: Coagulase (72 γ/ml.)
Lower graph: Calcium chloride
E. Activation of "low platelet" plasma by:
Upper graph: Thrombin (0.3 μ/ml.)
Middle graph: Coagulase (72 γ/ml.)
Lower graph: Calcium chloride

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023

TABLE 1.—*Clot Firmness*
Comparison of Coagulograph Maximum Amplitude (mm)

Activated by	Citrated Plasma	Fibrinogen Solution
Calcium	38	0
Thrombin 0.3 u/ml	2	15
Coagulase 72. γ/ml	7	1

TABLE 2.—*Clot Firmness*
Effect of Plasma Storage on Coagulograph Maximum Amplitude

Subject	Control	24 Hour Storage		2 Weeks Storage	
		Deep freeze −20C	Refrigerator +6C	Deep freeze −20C	Refrigerator +6C
H	64	35	60	30	27
B	71	39	65	39	39
M	49	25	32	21	15

ble for clot firmness. It is quite possible that a platelet factor may be present as a contaminant in "purified" fibrinogen preparations.

In contrast to thrombin, bacterial coagulase gives a more firm clot with plasma than with fibrinogen. This would be consistent with the concept of Tager[4] that bacterial coagulase activates the thrombin-producing mechanism. However, in our hands, coagulase caused rapid clotting of fibrinogen solutions which would not clot for hours or days after the addition of calcium and thromboplastin, suggesting that coagulase may act directly to convert fibrinogen to fibrin, or may markedly augment the activity of trace amounts of thrombin. Hemophilic plasma activated by coagulase gives a pattern indistinguishable from normal plasma similarly activated. Platelet count has little influence on the firmness of coagulase-activated plasma clots. Nevertheless, the fact that coagulase-activated plasma gives a firmer clot than coagulase-activated fibrinogen suggests that coagulase activity may involve interaction with plasma components (other than fibrinogen) which influence clot firmness.

Summary

1. The relative firmness of clots in different systems is not necessarily directly correlated with the speed of clot formation.

2. Thrombin will hasten the development of full clot firmness, but will not significantly alter the degree of clot firmness of citrated plasma specimens, although the firmness of "purified" fibrinogen preparations clotted by thrombin is increased with increasing thrombin concentration.

3. Citrate and/or calcium concentration influence clot firmness.

4. Fibrinogen preparations develop greater firmness on clotting with thrombin than do citrated plasma preparations.

5. A platelet factor influences clot firmness of recalcified plasma, but not of thrombin-activated or coagulase-activated plasma.

6. Clot firmness of recalcified citrated plasma is reduced by storage. This reduc-

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/514545/1125.pdf by guest on 01 February 2023

tion in firmness is more rapid if the specimen is deep frozen as compared to unfrozen aliquots at 6 C.

Summario in Interlingua

1. Le soliditate relative del coagulos in differente systemas non se trova necessarimente in correlation directe con le rapiditate del formation del coagulos.

2. Thrombina accelera le disveloppamento del complete soliditate del coagulo sed non altera significativemente le grado del soliditate del coagulo in specimens de plasma citrate, ben que le soliditate de preparatos de fibrinogeno "purificate" que es coagulate per thrombina se augmenta con le augmento del concentration de thrombina.

3. Le concentration de citrato e/o de calcium influentia le soliditate del coagulo.

4. Preparatos de fibrinogeno in comparation con citrate preparatos de plasma disveloppa plus alte grados de soliditate in coagulation con thrombina.

5. Un factor plachettal exerce un influentia super le soliditate del coagulo de plasma recalcificate sed non de plasma activate per thrombina o per coagulase.

6. Le soliditate del coagulo de citrate plasma recalcificate es reducite per immagasinage. Iste reduction del soliditate es plus rapide si le specimen es congelate a basse temperaturas in comparation con aliquotes non congelate e tenite a un temperatura de 6 C.

REFERENCES

[1] Hartert, H.: Die Thrombelastographie. Eine Methode zur physikalischen Analyse des Blutgerinnungsvorganges. Zeitschrift f. ges exper. Med. *117:* 189, 1951.

[2] Von Kaulla, K. N. and Weiner, M.: Studies of coagulation and fibrinolysis by new technic of continuous recording. Blood *10:* 362, 1955.

[3] Weiner, M. and Udenfriend, S.: The relationship of platelet serotonin to disturbances of clotting and hemostasis. Circulation *15:* 353, 1957.

[4] Tager, M.: Problems in the coagulation of plasma by staphylocoagulase. Ann. N. Y. Acad. Sc. *65:* 109, August 31, 1956.

Downloaded from http://ashpublications.org/blood/article-pdf/12/12/1125/51545/1125.pdf by guest on 01 February 2023