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THE GROUND-GLASS CLOTTING TIME

A Sensitive Screening Test for Blood Coagulation Defects

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A screening test should be simple to perform, give few false-negative results, and be little affected by factors other than those being tested. The whole-blood clotting tests currently in general use do not meet these criteria. While these tests detect most severe defects in blood coagulation, they are often normal in the presence of moderate or mild deficiencies. Thus, patients with mild hemorrhagic disorders may not be recognized from these tests, and yet during a surgical operation or after accidental injury serious bleeding may occur. Furthermore, the whole-blood clotting tests are too insensitive to be of value in monitoring the results of replacement therapy in, for instance, a patient with hemophilia. A quick, reliable, and sensitive test is needed.

Since 1959 we have been using a test in which increased surface activation of the coagulation mechanism is achieved by the addition of fragments of glass. We have called this test the *ground-glass clotting time*. It is extremely simple to perform, can be carried out quickly at the bedside and is little affected by variations in technic. Furthermore, it has proved to be much more sensitive than the Lee and White[1] clotting test and slightly more sensitive than the partial thromboplastin time (PTT).[2]

This report evaluates the ground-glass clotting time (GGCT) as a screening test for hemorrhagic disorders and as a monitor for coagulant and anticoagulant therapy.

Materials and Methods

The particles of glass used for the ground-glass clotting time are obtained by crushing discarded glassware using a mortar and pestle. The resulting

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fragments vary in size from fine dust to particles about 1 mm. in diameter. Finely powdered glass, such as is available commercially, is not satisfactory because the powder tends to form into a single cake and does not mix well with the blood. Ground glass (about 75 mg.) to cover the bottom curvature is placed in each of three 10 by 75 mm. glass test tubes. The amount of glass may be doubled or halved without affecting the results. The three test tubes are placed in a heating block set at 37 C. Blood is drawn from a vein into a plain glass syringe, and approximately 1 ml. is transferred into each of the three test tubes. These tubes are quickly and firmly sealed with size 000 rubber stoppers. The first tube is immediately inverted and a stopwatch is started. The three tubes, in rotation, are then rapidly inverted until a clot appears. The end point is the formation of a solid clot that adheres to the bottom of the test tube. The change from liquid blood to a firm clot occurs rapidly between one or two tiltings. When the clotting time is considerably prolonged, the end point may be noted as the formation of a large clot that slides down the tube when it is inverted. The clotting time of each tube is noted to the nearest 5 seconds, and the average of the three results is reported as the ground-glass clotting time.

The modification of the Lee and White test,[1] which is in use in our laboratory, is performed as follows. Venous blood is collected in a plain glass syringe and approximately 1 ml. is placed into each of three plain 10 by 75 mm. glass test tubes maintained at 37 C. The tubes are left undisturbed for 5 minutes and then the first tube is tilted every 30 seconds until a clot has formed. The blood is considered to have clotted if it fails to flow when the tube can be gently tilted to the horizontal. The second tube in turn is then tilted until the blood has clotted. Finally, the third tube is tilted every 30 seconds until the blood has clotted. The time taken for the last tube to clot is reported as the Lee and White clotting time.

The partial thromboplastin test[2] was performed using Thrombofax.* The plasma used in this test was prepared from a mixture of 1 ml. of a 1.4 percent sodium oxalate solution and 5 ml. of venous blood.

The thromboplastin generation test (TGT) was performed as described by Biggs and Douglas.[3] Cephaloplastin† was used on occasions as a substitute for platelets in the incubation mixture. Factor VIII (AHF) was assayed either in the TGT or the PTT. In either case, a reference curve was prepared by measuring the ability of various saline dilutions of adsorbed pooled normal plasma to correct the thromboplastin generation of known hemophiliac plasma. At least two dilutions of adsorbed test plasma were assayed and the percentage of factor VIII was derived by comparison of the results with the standard curve.

* *Ortho Pharmaceutical Corporation.*
† *Dade Reagents, Inc.*

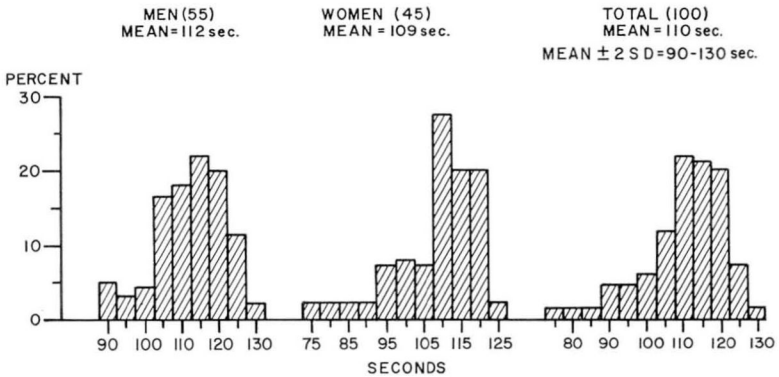

Fig. 1. The ground-glass clotting time of 100 normal persons (55 men and 45 women).

Table 1.—*Comparison of the Lee and White clotting time (LW) and ground-glass clotting time (GGCT) in various hemorrhagic disorders*

Disorder	Patients, number	Tests, number		
		Total	Both abnormal	Normal LW and abnormal GGCT
Hemophilia	18	30	24	6
Christmas disease	5	13	9	4
Hypofibrinogenemia	3	6	1	4
Circulating anticoagulant	8	28	18	10
Total	34	77	52	24

RESULTS

The ground-glass clotting time of 100 healthy adults is shown in *Figure 1*. The normal range is from 90 to 130 seconds. When the test is performed at room temperature, the normal range extends up to 150 seconds and some reproducibility is lost. Within the normal range, there is seldom more than 10 seconds between the clotting time of the first tube and that of the third tube. This difference increases slightly when the clotting time is prolonged. Doubling or halving the quantity of ground glass has no detectable effect on the results.

The normal range for the Lee and White clotting time is up to 20 minutes, and that for the partial thromboplastin time is from 60 to 100 seconds.

Table 1 summarizes the comparison between the Lee and White and the

Table 2.—*Comparison of the Lee and White clotting time (LW) and ground-glass clotting time (GGCT) of blood from patients with disorders of platelets*

Platelet disorder	Patients, number	Tests, number			
		Total	Both normal	Both abnormal	Normal LW and abnormal GGCT
Thrombocytopenia	6	7	1	0	6
Thrombocytopathy	18	25	5	11	10

ground-glass clotting times in four groups of patients with proved hemorrhagic defects. In blood from one third of these patients, the Lee and White clotting time was normal, whereas the ground-glass clotting time was abnormal in blood from all patients, except one with hypofibrinogenemia. The group of patients with circulating anticoagulants includes five patients treated with heparin and three patients whose plasma contained anti-factor VIII. Three patients with factor VII deficiency are not included in *Table 1;* the ground-glass clotting time and the Lee and White clotting time were normal in all three patients.

The results shown in *Table 2* demonstrate that the ground-glass clotting time was prolonged in blood from a significant number of a small group of patients with thrombopenia. The Lee and White clotting time was normal in all instances. The ground-glass clotting time was also abnormal in blood from a number of patients with various types of thrombocytopathy, involving abnormalities in content or release of platelet factor 3. The majority of these patients each had an acquired defect associated either with severe uremia or with polycythemia vera.

Table 3 shows the correlation between the plasma factor VIII content, the ground-glass clotting time, and the partial thromboplastin time, for a group of hemophiliacs who received transfusions of fresh frozen plasma, or concentrates of factor VIII. The ground-glass clotting time was prolonged when the factor VIII content was between 20 and 25 percent; however, the PTT was normal at this level.

DISCUSSION

The clotting time of whole blood in a glass test tube depends on many factors, but, in all probability the amount of contact between the blood and the foreign surface accounts for most of the differences between various methods. There are two ways in which the amount of contact may be standardized. One method is to reduce contact to a minimum by using siliconized

Table 3.—*Comparison of the results of the partial thromboplastin test (PTT) and the ground-glass clotting time (GGCT) at various values of factor VIII. The patients were hemophiliacs receiving plasma or factor VIII concentrate.*

Range of factor VIII (AHF), percent	Test	
	GGCT, sec.	PTT, sec.
0–5	540	115
	450	240
	480	160
	260	205
	295	92
10–15	280	125
	155	140
	240	90
	190	90
	185	114
20–25	200	96
	210	81
	185	85

glassware or other nonwettable plastic material. Methods based on this principle produce normal clotting times in the range of 45 to 60 minutes. It might be expected that the difference between normal and abnormal would be accentuated with such a long normal clotting time, but the gray zone between definitely normal and abnormal is proportionately widened and little increase in sensitivity is obtained. Using this method, clotting begins as a fine, fibrin film at the air-blood interface and spreads slowly through the specimen. This makes it difficult to determine the end point, and leads to considerable variation between the results recorded by different observers. The time taken to perform the test, especially if the result is abnormal, is a further disadvantage. The second method is to provide maximal contact, as we have done in the ground-glass clotting time. The advantages of this approach are its speed, the sharp end point, and the reproducibility of results obtained by different observers.

Whatever variation of the basic Lee and White technic is used, normal results are obtained for a few patients with severe and for many with mild coagulation defects.[4, 5] We found normal Lee and White clotting times in 3 of 18 patients with severe hemophilia. The Lee and White clotting time and its various modifications are so unreliable that their use is difficult to justify. It is quite possible to be lulled into a false sense of security by a normal result.

The most frequently suggested alternative screening procedures of blood coagulation have been a detailed history and the partial thromboplastin test. A thorough investigation of the clotting mechanism should be carried out in any patient whose personal or family history suggests a hemorrhagic tendency, regardless of the results of the screening tests. The partial thromboplastin test is, in essence, a plasma clotting time with a platelet substitute added to the mixture. There is no doubt that it is a good screening test and a great improvement on the Lee and White test. However, it does have a few disadvantages. To perform a partial thromboplastin test the blood requires some processing that precludes determination at the bedside, whereas the ground-glass clotting time can be performed at the bedside and promptly provide an answer. When an answer is not required in a short time, the partial thromboplastin test has the advantage that it can be performed, and repeated, at leisure, and that it can be used for correction studies. Furthermore, the partial thromboplastin test can be used as the basis of a reliable factor VIII assay. However, when used as a screening test, in our experience it is abnormal only when the plasma factor VIII level is less than 20 percent; whereas the ground-glass clotting time remains abnormal up to 25 percent levels of factor VIII. The partial thromboplastin time was normal in one patient who had Christmas disease with 10 percent factor IX, while the ground-glass clotting time was 170 seconds. The ground-glass clotting time was normal in three patients with hereditary factor VII deficiency, as were all other tests of the intrinsic pathway of blood coagulation.

The abnormal ground-glass clotting time found in some patients with thrombopenia requires some explanation, because it is generally considered that the clotting time is normal under these circumstances. It is possible that when the coagulation system is speeded up, as it is in the presence of nearly maximum contact, the number of platelets may become a limiting factor. The ground-glass clotting time was also abnormal in 80 percent of patients with a thrombocytopathy due to failure to release factor 3.

Several foreign additives have been used in various tests of blood coagulation. Margolis[6] found that the addition of kaolin to plasma in the recalcified plasma clotting time increased the sensitivity considerably. More recently, various activators have been used in an attempt to improve the partial thromboplastin test. It seems likely that all these substances expedite the intrinsic coagulation system by activating factor XII.[7] When applied to the whole-blood clotting time, we have found that particles either of ground glass or of kaolin are suitable. We use ground glass in preference to kaolin because we have had greater experience with it.

When the ground-glass clotting time, the Lee and White clotting time and the partial thromboplastin time are compared as screening tests (*Ta*

Table 4.—*A rating of the ground-glass clotting time (GGCT), the Lee and White clotting time (LW), and the partial thromboplastin time (PTT) as screening tests*

Criteria	Rating		
	GGCT	LW	PTT
Simplicity	Excellent	Fair	Fair
Reproducibility	Good	Poor	Excellent
Sensitivity	Excellent	Poor	Good

ble 4), we find the ground-glass clotting time to be superior in all aspects except reproducibility, in which the partial thromboplastin time holds a slight advantage.

SUMMARY

A new method for measuring the clotting time of whole blood, in which glass particles provide added surface activation, is described. This ground-glass clotting time has a normal range of from 90 to 130 seconds with a clear-cut end point. It is reproducible, varies little with minor differences in technic, and may be performed at the patient's bedside. It is sensitive, being abnormal when the plasma factor VIII content is as high as 25 percent, a level at which the partial thromboplastin time is normal. The ground-glass clotting time is, in our experience, the most useful screening test for detecting abnormalities of blood coagulation. It has also proved to be a sensitive monitor during replacement therapy of such disorders.

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