A TEST FOR INCREASED COAGULABILITY OF THE BLOOD*

By Theo R. Waugh, M.D. and Capt. D. W. Ruddick, R.C.A.M.C.

Montreal

FOR many years clinical interest in the coagulability of the blood has concerned itself, almost entirely, with the hæmorrhagic diatheses and abnormalities of one kind or another leading to a prolongation of the process of clotting. More recently, however, with the discovery of anticoagulants that can be employed therapeutically, such as heparin and dicoumarin, great interest has arisen in the subject of thrombosis and the relation of an increased coagulability of the blood to the development of this condition.

Unfortunately, the various tests that have been developed for measuring the clotting time, while satisfactory for demonstrating a prolongation of the process, do not allow for accurate measurement when acceleration occurs. This is due principally to the fact that the time under normal conditions is relatively short and unless influencing factors are very carefully controlled they are quite likely to accelerate the process to such a degree that the results are not dependable. These objections hold not only for those tests that are dependent upon the initial formation of fibrin for their end point, such as the use of a capillary tube, Bürker's method, the Boggs coagulometer, etc., but also for the congealing method of Lee and White. In all of these tests there is a comparatively wide range between normal limits. There would appear to be, therefore, an immediate demand for a method that would allow for much finer analysis of any shortening of the clotting time.

In an attempt to develop such a method, after a considerable amount of experimentation with various possibilities, it occurred to us that this might be accomplished by a controlled deceleration of the process. That is, if the coagulation could be put into "slow motion" as it were, finer changes would be magnified and thus more accurately measured. This, in fact, was found possible through the use of heparin, which apparently exerts its inhibitory effect by counteracting the action of thromboplastin. By this means, therefore, an indirect measurement of the thromboplastin content of the blood is obtained and, as theoretically an increase in the amount of this substance is the cause of accelerated coagulation, the desired result was achieved.

After trying the use of heparin in various quantities and different coagulation tests, and studying the effects of temperature, humidity, etc., on the process, it appeared that the congealing test of Lee and White lent itself most readily to modification and adaptation to our purpose and the method, the details of which are given below, was devised.

TECHNIQUE

The heparin used is that obtained from the Connaught Laboratories and having a potency of 1,000 units per c.c. Six-tenths of a cubic centimeter of heparin (600 units) are aseptically withdrawn from the vial, using a 1 c.c. tuberculin syringe to which a 22 gauge needle has been attached. This amount of heparin is then added to 300 c.c. of physiological (0.9%) saline. This is mixed thoroughly and results in a fluid containing two units of heparin per c.c. of saline. This is a stock solution. Eight small glass-stoppered bottles with large mouths (capacity 60 c.c.) are cleaned thoroughly, and subdilutions from the stock solution are made in them in the following manner:

1. Five c.c. of stock solution (containing ten units heparin) are added to 45 c.c. of saline, resulting in a sub-dilution containing 10 units of heparin per 50 c.c. of saline. This bottle is then labelled, No. 1, 1/10 unit per ½ c.c.

2. Ten c.c. of stock solution (20 units heparin) are added to 40 c.c. of saline. This is then labelled, No. 2, 2/10 units per ½ c.c.

3. Fifteen c.c. of stock solution are added to 35 c.c. normal saline and labelled No. 3, 3/10 units per ½ c.c.

In each subsequent bottle the amount of stock solution is increased by 5 c.c., and the saline is decreased by 5 c.c. until in the seventh bottle 35 c.c. of stock solution are added to 15 c.c. of saline. This procedure results in seven bottles containing concentrations of heparin which increase by 1/10 unit of heparin per ½ c.c. of saline in each bottle. The eighth bottle is then filled with saline to be used as a control.

This prepares sufficient fluid dilutions for at least 100 tests and when not in use the bottles,

* From the Departments of Pathology of the Royal Victoria Hospital and of McGill University.

Aided by grant from the Associate Committee on Army Medical Research, National Research Council of Canada.

as well as the stock solution, should be kept in a refrigerator so as to maintain the potency of the heparin.

Nine Wassermann tubes (100 mm. x 13 mm. outside diameter) thoroughly cleaned and dried are placed in a special test-tube rack which has been constructed so that it rotates about a central axis (Fig. 1). The proper cleaning of the tubes is important. They should be treated with potassium dichromate-sulphuric acid solution, rinsed in hot water to remove all cleansing fluid, then after rinsing in distilled water, dried thoroughly with gauze. Using a one-half cubic centimeter or finely graduated pipette the fluids are placed in the test tubes as follows.

No. 1 tube (left hand side) is left empty.

No. 2 tube receives one-half c.c. normal saline (control).

No. 3 tube receives ½ c.c. heparin dilution from bottle No. 1, *i.e.*, 1/10 unit heparin per ½ c.c. saline.

No. 4 tube receives one-half c.c. heparin dilution from bottle No. 2. This procedure is continued, using corresponding heparin dilutions until tube No. 9 contains 7/10 units heparin per ½ c.c. saline.

The end result therefore is as follows:

Tube No.	*Contents*
1.	empty
2.	½ c.c. normal saline
3.	½ c.c. normal saline + 1/10 unit heparin
4.	½ c.c. normal saline + 2/10 units heparin
5.	½ c.c. normal saline + 3/10 units heparin
6.	½ c.c. normal saline + 4/10 units heparin
7.	½ c.c. normal saline + 5/10 units heparin
8.	½ c.c. normal saline + 6/10 units heparin
9.	½ c.c. normal saline + 7/10 units heparin

Corks are placed in each tube until the blood is to be added. Using a dry 20 or 30 c.c. graduated Luer syringe and a large gauge (18) needle, ten to twelve c.c. of blood are withdrawn from the arm vein of the patient. One c.c. of this blood is placed in each test tube which is then recorked. If the needle is left on, this can be measured directly from the syringe. Then the rack is agitated slightly so as to insure complete mixing of the fluid and the blood in the tubes. The time is then recorded and the actual test which is conducted at room temperature begins. The test tube rack is gently rotated in a clockwise direction every 2 minutes to an angle of 70 to 80° and the fluidity of the blood in each tube is noted (Fig. 2). The end point for each tube (congealing) is that point at which the blood no longer flows down the side

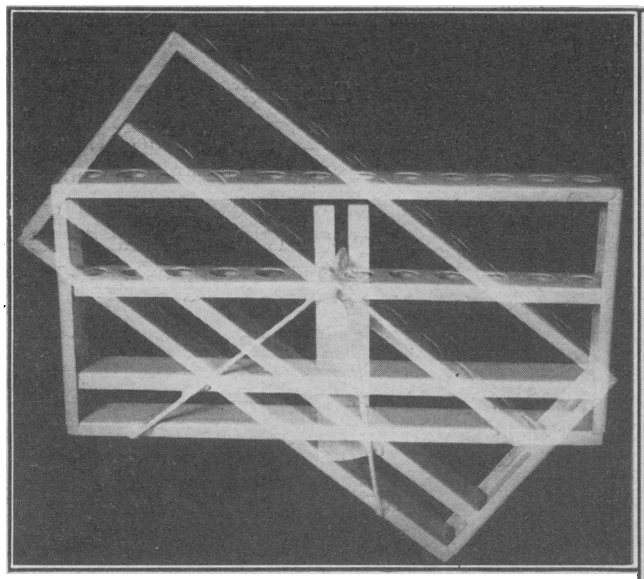

Fig. 1

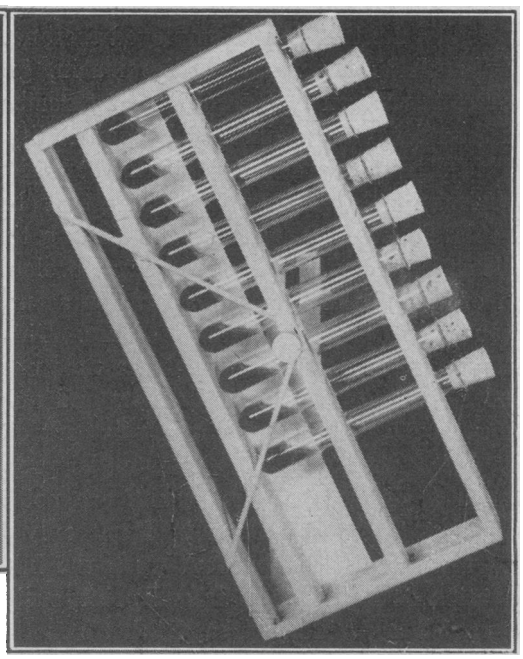

Fig. 2

Fig. 1.—Empty rack showing construction for rotation on central axis (double exposure).

Fig. 2.—Rack with tubes in rotated position. Congealing has occurred in tubes 1 to 5, but blood is still fluid in tubes 6 to 9.

of the test tube (modified Lee and White method).

Some difficulty may be experienced in determining the end point by those unfamiliar with the test. This is due, in part, to a fine membrane forming on the surface of the blood, which corking of the tubes helps to prevent. This membrane can be broken by rotating the test tube rack to the desired angle and maintaining this angle for 10 to 15 seconds. Another factor which may cause erroneous reading is that small deposits of fibrin sometimes appear on the surface of the tube over which the blood has flowed. This will disturb the smooth flow of blood and

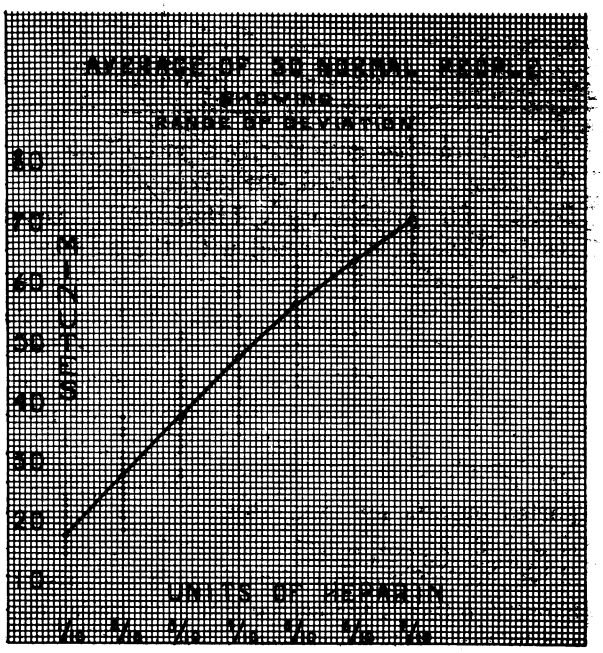

Fig. 3.—Graph showing results of test on fifty normal adults. The mathematical average is expressed by the continuous dark line.

can be prevented by rotating the individual tube in question in the rack or by rotating the rack in a counterclockwise direction thus allowing the blood to flow over a smooth clean surface on the opposite side of the tube. No. 1 tube congeals first in 5 to 11 minutes. This is followed by tube No. 2, then No. 3, etc., until the blood in every tube has congealed. The whole test usually requires approximately one hour. The time taken for each tube to congeal is then recorded on graph paper, plotting 1/10 units heparin against time in minutes.

RESULTS IN NORMAL INDIVIDUALS

In order to establish the range of deviation of the graph using this test on normal individuals, fifty adults, ranging in age from 18 to 60 years and of both sexes were examined. The composite results are demonstrated in Fig. 3, with the mathematical average expressed as a continuous dark line. The shortest and longest times of congealing for each heparinized tube, together with the average time for the fifty individuals, are as follows:

	Shortest duration	*Average time*	*Longest duration*
Tube No. 3	10 mins.	18 mins.	25 mins.
Tube No. 4	19 "	28 "	38 "
Tube No. 5	29 "	38 "	52 "
Tube No. 6	35 "	48 "	60 "
Tube No. 7	43 "	57 "	70 "
Tube No. 8	52 "	64 "	83 "
Tube No. 9	64 "	71 "	88 "

This series of tests shows that while there is a certain amount of variation in normal adults the graph falls within quite well defined limits and these were therefore established as the bounds of normalcy. Any graph, the curve of which falls below these limits indicates an accelerated coagulability; while a slowing of the coagulation time produces the opposite effect. Occasionally the reading on a single tube will not fit into the general contour of the curve of the graph and should be neglected, as it is obviously due to an extraneous factor. If, however, the graph because of gross irregularity fails to demonstrate any definite impression of the velocity of coagulation, the test should be repeated.

We have now used this method for some time on clinical cases and have found that it demonstrates very satisfactorily an increased coagulability of the blood. Preliminary studies have shown that an acceleration usually is present (1) during uncomplicated bed rest; (2) following operative procedures, and (3) in the presence of acute infections. Many interesting observations of changes in coagulability have been observed in individual cases. These results, together with studies dealing with theoretical features of the test, will be reported in a subsequent paper.

SUMMARY

The detailed technique of a method that allows for the demonstration of an increased coagulability of the blood is presented.