

# LABORATORY TESTS FOR INCIPIENT THROMBOSIS

BY

G. I. C. INGRAM\* AND ROSEMARY BIGGS

with analyses by

P. ARMITAGE

*From the Department of Surgery, University of Edinburgh, the Department of Pathology, Radcliffe Infirmary, Oxford, and the Medical Research Council Statistical Research Unit, London School of Hygiene and Tropical Medicine*

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The use of anticoagulant therapy in the treatment of thrombosis is now widespread, and there is little doubt of its value. It is therefore not surprising that many laboratory workers have attempted to find tests to demonstrate hypercoagulability of the blood, so that, in susceptible cases, anticoagulants might be used to prevent the onset of thrombosis.

It appears that blood does not clot solid in the vessels of normal people because the surface with which it is in contact does not readily liberate the thromboplastin which is essential for the conversion of prothrombin to thrombin. When blood is withdrawn from the body and maintained at 37° C. in a glass tube, clotting occurs in five to 10 minutes because contact of blood with the glass surface leads to the liberation of a powerful thromboplastin. After coagulation of normal blood in a glass tube most of the prothrombin activity disappears from the serum; the extent to which this has occurred may be an index of the efficiency of the blood thromboplastin system. When blood is held at 37° C. in tubes coated with silicone, it takes upwards of 20 minutes to clot and only a fraction of the prothrombin activity disappears: it is thought that this is because the silicone surface does not readily promote the liberation of thromboplastin. Thus it is probably true to say that clotting of normal blood is limited by the extent to which free, active thromboplastin can be formed. The diagnosis of a "prethrombotic state" may therefore depend on demonstrating some abnormality of the blood thromboplastin system. Since there were at the time when these investigations were made no specific tests for the investigation of this system, indirect methods were used.

Three techniques are available. The first method is to compare the clotting time of whole unaltered

blood in vessels of different surfaces. The second method is to study the extent to which prothrombin is converted to thrombin during clotting, especially during clotting in siliconed vessels. The third method is to measure the reaction of blood to heparin; this substance delays the conversion of prothrombin to thrombin and also inhibits the thrombin-fibrinogen reaction.

It has been claimed that, in patients who develop thrombosis, the whole blood clotting time (Cummine and Lyons, 1948; Barker and Margulies, 1949) and the results of the heparin "resistance" test (de Takáts, 1943; de Takáts and Fowler, 1945) are abnormal. Since the clinical problem is an important one, it was thought worth while to study these tests once more, and, in addition, to investigate the prothrombin consumption of blood clotting in both glass and silicone-treated tubes.

## Methods

**Venepuncture.**—With a tourniquet applied proximally, blood was withdrawn from an antecubital vein into a dry, paraffined syringe: neither syringe nor needle was treated with silicone.

**Reagents.**—The following reagents were used: human brain thromboplastin (Biggs and Macfarlane, 1949); purified human fibrinogen solution (Jaques, 1943); calcium chloride, M-40 aqueous solution; sodium citrate, 3.8% aqueous solution.

**Techniques.**—The techniques used were as follows:

**Whole Blood Clotting Time.**—This was determined by (1) the method of Lee and White (1913) in which 1.0 ml. blood was delivered from the syringe into (normally) four 7.5 × 0.9 cm. glass tubes in a water-bath at 37° C.: the tubes were tipped at half-minute intervals until blood did not run on inversion: clotting was timed from the first flow of blood into the syringe. The mean clotting time of the four replicates was recorded. (2) The determination was made as in (1), with the tubes silicone-coated (Jaques, Fidler, Feldsted, and Macdonald, 1946a, b). (3) The

\* Member of external staff, Medical Research Council.

method of Dale and Laidlaw (1911-12) in which capillary blood was run into bead-capillaries measuring  $25 \times 1.5$  mm.: the ends were occluded and the tubes were tipped to and fro in the water-bath at  $37^{\circ}$  C. until the bead was arrested: clotting was timed from the skin puncture. Usually a single test was made, but sometimes replicates were made from two different punctures, in which case the mean clotting time was taken as the response.

**"Prothrombin Consumption"** (Quick, 1947).—About 50 minutes after venepuncture the clots in the eight Lee and White tubes were loosened when necessary, and the sera obtained about 10 minutes later by centrifugation were pooled from the two series of tubes into two glass tubes held at  $37^{\circ}$  C. Two hours after venepuncture 0.1 ml. samples from each tube were added to 0.1 ml. aliquots of fibrinogen solution, followed by 0.1 ml. of thromboplastin and 0.1 ml. of calcium chloride solutions, clotting time being measured from the last addition. (In a few instances a two-hour reading was not made, but the mean of readings at one and three hours was used instead.) As a control of the reagents, particularly the fibrinogen, which occasionally appeared to inhibit the reaction, the test was repeated with 0.1 ml. of the patient's citrated plasma, spun off from citrated blood (see below) in place of the serum. If a plasma clotting time of over 30 seconds was obtained, fresh reagents were prepared for the tests on the sera.

**"Heparin Resistance" Test** (Vaughn and Ruddick, 1944a, b).—Saline solutions of heparin were prepared in the series 0.2, 0.4, 0.6 . . . 2.0 units per ml., and 0.5 ml. of each placed in  $7.5 \times 0.9$  cm. glass tubes in the  $37^{\circ}$  C. water-bath. Initially 1.0 ml. of blood was added to each tube directly from the syringe, but later *citrated blood* (1.0 ml. sodium citrate solution to 9.0 ml. blood) was used and the heparin solutions calcified; in this technique the tube series was usually reduced to the six lowest concentrations only. The clotting times were determined as in the Lee and White tests. The reacting heparin concentrations were determined by calculating the total fluid volume in the tubes from the haematocrit of the citrated blood. Haematocrit values were retrospectively estimated for the analysis of certain early cases by reference to the clinical details recorded, as measurements had not been made in these instances (the error introduced was small compared with differences between persons). One to four saline control tubes were also tested with the heparin tubes.

### Subjects

Three clinical groups of subjects were tested: normal controls, abnormal controls, and thrombotics. The clinical material was obtained in the wards of the Surgical Professorial Unit at the Royal Infirmary, Edinburgh, and in various wards of the Radcliffe Infirmary, Oxford, where the techniques were initially standardized.

Not all subjects were investigated by all the tests: the numbers are given in the tables. Individuals re-tested either in the same clinical group at intervals of several months or in more than one clinical group counted each time as a separate subject, but when patients were re-tested after intervals of a few days, while the clinical condition was materially unaltered, the duplicated readings were pooled. Some 15 to 20 individuals were tested twice (the degree of overlapping between the normal subgroups is only approximately known), the readings from five of whom were pooled, and one subject was tested three times.

**Normal Controls.**—Blood was obtained from laboratory staff, from ex-patients at follow-up, from patients' relatives, and from apparently healthy persons undergoing routine tests for blood grouping and serology. These controls fell into two subgroups. Subgroup 1 consisted of 32 subjects tested concurrently with the abnormal and the thrombotic subjects, and by the same observers. Subgroup 2 consisted of 50 subjects tested at a different time, and to some extent by different observers in the same laboratory. The whole blood clotting time was measured by the method of Dale and Laidlaw on all the subjects, and by that of Lee and White on 36 subjects. A comparison between the two subgroups thus provides a measure of differences that may be expected in the results in these two well-established tests when made on normal persons under slightly different conditions.

**Abnormal Controls.**—Tests were made on medical and surgical bed patients and on maternity cases shortly before or after delivery, none of whom was believed to have had thrombosis at the time of testing: there were in all 40 patients.

Three patients with thrombocytopenia were also tested by the whole blood clotting time and heparin resistance tests, as a control of the sensitivity of the latter in demonstrating the change known to be found in this condition. They were all tested against heparin with citrated blood.

**Thrombotic Cases.**—Forty subjects of various clinical types were tested: the method of classification of the material is shown in Table II. As far as possible tests were made before anticoagulant treatment was begun: details of the exceptions are given in Table II. The other tables give the results for each test separately by broad clinical groups; in these tables the thrombotic cases have been subdivided so that thrombotic subgroup 1 (gross, acute venous thrombosis) is shown

separately, the remaining thrombotic subgroups having been merged.

**Selection of Cases.**—For abnormal controls tests were made either at random on bed patients or on those patients from whom blood happened to be required for biochemical investigations. All available cases of thrombosis were tested with the exception of those in which the diagnosis seemed very doubtful or in which anticoagulant treatment

was well established, together with a small number of instances in which a "clean" venepuncture could not be performed.

## Results

**Presentation.**—The complete data are not given, but the distribution of test results in each clinical group is summarized by showing the mean with its standard error and, in Tables I and III, with

TABLE I

WHOLE BLOOD CLOTTING TIMES FROM CONTROLS AND PATIENTS AND THROMBOTICS BY MAIN CLINICAL GROUPS

Clinical Group	No. of Subjects Tested, Mean and Standard Error with Standard Deviation for Each Test (min.)								
	Lee and White (Glass)			Lee and White (Silicone)			Dale and Laidlaw		
	<i>n</i>	Mean $\pm$ S.E.	S.D.	<i>n</i>	Mean $\pm$ S.E.	S.D.	<i>n</i>	Mean $\pm$ S.E.	S.D.
Normal controls:									
Subgroup 1 .. .. .	17	7.8 $\pm$ 0.4	1.5	17	20.1 $\pm$ 1.4	5.9	16	2.14 $\pm$ 0.12	0.46
" 2 .. .. .	36	6.2 $\pm$ 0.2	0.9	—	—	—	50	2.13 $\pm$ 0.04	0.26
Abnormal controls .. .. .	24	9.2 $\pm$ 0.4	2.2	18	20.4 $\pm$ 1.7	7.4	22	2.25 $\pm$ 0.12	0.56
Thrombotics:									
Subgroup 1 .. .. .	7	8.9 $\pm$ 0.5	1.2	5	17.0 $\pm$ 3.3	7.4	6	2.08 $\pm$ 0.13	0.31
Subgroups 2-6 .. .. .	33	9.1 $\pm$ 0.3	1.5	30	22.1 $\pm$ 1.7	9.3	27	2.00 $\pm$ 0.08	0.43

S.E. = Standard error of the mean. S.D. = Standard deviation. *n* = Number of subjects tested.

TABLE II

WHOLE BLOOD CLOTTING TIMES AND "HEPARIN RESISTANCE" TEST DATA FROM 40 THROMBOTIC PATIENTS ANALYSED BY CLINICAL SUBGROUPS

Clinical Subgroup	Total No. of Subjects	No. of Observations, Mean, and Standard Error of Mean for Each Test									
		Whole Blood Clotting Time (min.)						"Heparin Resistance"			
		Lee-White (Glass)		Lee-White (Silicone)		Dale-Laidlaw		Slope (min. u.)		Slope/Saline Control	
		<i>n</i>	Mean $\pm$ S.E.	<i>n</i>	Mean $\pm$ S.E.	<i>n</i>	Mean $\pm$ S.E.	<i>n</i>	Mean $\pm$ S.E.	<i>n</i>	Mean $\pm$ S.E.
1. <i>Gross acute venous thrombosis</i> .. .. .	7										
Tested on day of clinical onset .. .. .	2	2	7.8 $\pm$ 1.1	2	20.3 $\pm$ 7.1	1	1.88 $\pm$ 0.43	2	19.5 $\pm$ 7.4	2	6.5 $\pm$ 2.4
" " first day after clinical onset .. .. .	2	2	10.0 $\pm$ 1.1	1	20.4 $\pm$ 10.0	2	1.92 $\pm$ 0.31	1	46.9 $\pm$ 10.5	1	9.2 $\pm$ 3.3
" " second " " " " .. .. .	2	2	9.2 $\pm$ 1.1	1	11.0 $\pm$ 10.0	2	2.38 $\pm$ 0.31	1	31.6 $\pm$ 10.5	1	15.8 $\pm$ 3.3
" " third " " " " .. .. .	1	1	8.3 $\pm$ 1.6	1	13.2 $\pm$ 10.0	1	2.00 $\pm$ 0.43	1	23.8 $\pm$ 10.5	1	4.5 $\pm$ 3.3
2. <i>Minor acute venous thrombosis treated within a week of clinical onset</i> .. .. .	9										
Superficial, at site of intravenous infusion .. .. .	3	3	10.4 $\pm$ 0.9	3	23.2 $\pm$ 5.8	3	1.83 $\pm$ 0.25	3	18.6 $\pm$ 6.1	3	4.2 $\pm$ 1.9
Recumbency thrombosis of leg without gross venous obstruction .. .. .	6 <sup>a, a, b</sup>	6	9.0 $\pm$ 0.7	4	27.2 $\pm$ 5.0	6	2.21 $\pm$ 0.18	4	18.5 $\pm$ 5.3	4	4.7 $\pm$ 1.7
3. <i>Other recent thrombosis</i> (duration before testing, or extent, uncertain) .. .. .	4 <sup>f</sup>	4	8.8 $\pm$ 0.8	4	25.1 $\pm$ 5.0	2	2.09 $\pm$ 0.31	4	24.6 $\pm$ 5.3	4	8.3 $\pm$ 1.7
4. <i>History of multiple thromboses</i> (no evidence of thrombosis when tested) .. .. .	2 <sup>d</sup>	2	7.7 $\pm$ 1.1	2	24.2 $\pm$ 7.1	2	1.67 $\pm$ 0.31	2	20.2 $\pm$ 7.4	2	7.8 $\pm$ 2.4
5. <i>History of a single thrombosis</i> (three or more weeks before testing; no evidence of a thrombosis when tested) .. .. .	7 <sup>b, f, f</sup>	7	8.9 $\pm$ 0.6	6	19.6 $\pm$ 4.1	5	1.91 $\pm$ 0.19	7	33.9 $\pm$ 4.0	7	7.4 $\pm$ 1.2
6. <i>Occlusive arterial disease</i> .. .. .	11										
Young patients with migratory phlebitis or with histological evidence of thromboangiitis obliterans .. .. .	6	6	9.5 $\pm$ 0.7	6	21.2 $\pm$ 4.1	5	1.87 $\pm$ 0.19	6	25.4 $\pm$ 4.3	6	5.9 $\pm$ 1.4
Other patients .. .. .	5 <sup>c, e</sup>	5	9.2 $\pm$ 0.7	5	18.3 $\pm$ 4.5	4	2.23 $\pm$ 0.22	5	28.0 $\pm$ 4.7	5	6.2 $\pm$ 1.5

Details of anticoagulants exhibited in those patients already under treatment at time of testing (each letter stands for one patient in the group indicated: other patients untreated at time of testing): (a) Patient on heparin; tests made 4-5 hr. after last intravenous injection. (b) Patient on heparin; tests made 20 hr. after last intravenous injection. (c) Patient receiving salicylates (as analgesics): "prothrombin time" unrecorded. (d) Patient receiving salicylates (as analgesics): "prothrombin time" normal (i.e. = control time). (e) Patient receiving salicylates (as analgesics): "prothrombin time" prolonged (control 15.5 sec.; patient 17 sec.; 1-stage venom technique). (f) Details unrecorded. The standard error attached to each mean is obtained from the pooled mean square within the eleven small subgroups.

TABLE III  
 "PROTHROMBIN CONSUMPTION" TEST\* (SILICONE SERA) FROM CONTROLS AND PATIENTS AND THROMBOTICS ANALYSED BY MAIN CLINICAL GROUPS

Clinical Group	No. of Subjects	Mean $\pm$ S.E.	S.D.
Normal controls (subgroup 1)	14	26.8 $\pm$ 3.3	12.3
Abnormal controls .. ..	16	28.1 $\pm$ 2.7	10.7
Thrombotics:			
Subgroup 1 .. ..	4	33.0 $\pm$ 9.6	19.2
Subgroups 2-6 .. ..	21	29.8 $\pm$ 3.5	15.9

\*This test was not applied to the sera from the thrombocytopenic patients. Clotting time is given in secs.

the standard deviation; comparisons of test results are confined to the means because some distributions were significantly skew.

**Sex and Seasonal Variations.**—A preliminary analysis of the results obtained from normal persons, the only group from which suitable data were available, showed that the sex or the seasonal differences (October–March vs. April–September) were not significant for any test. These factors have been ignored in the subsequent analysis.

**Clotting Time of Venous Blood in Glass Tubes (Lee and White Method).**—This test was made on 53 normal subjects, on 24 patients who did not develop thrombosis but were in bed in hospital, and on 40 thrombotic patients. The results are given in Tables I and II. Table I shows the comparisons between the broad clinical groups, and in Table II the statistics are given for each thrombotic subgroup.

The two subgroups of normal subjects gave mean values which were found to be significantly different: this difference must be ascribed to undetected differences in the conditions of testing. The abnormal controls had a higher mean value than either of the normal control groups, and again the differences were significant. These results illustrate the difficulty of applying such tests to the study of any but large differences between patients. It must be supposed that in obtaining the results from the two groups of normal subjects the endpoints were read in a slightly different way or that some other unidentified factor influenced the results.

There was no significant difference between the abnormal control group and the patients with thrombosis (Table I).

**Clotting Time of Venous Blood in Silicone Tubes (Lee and White Method).**—Barker and Margulies (1949) have claimed that the whole blood clotting time of patients with thrombosis may be shorter than normal if the tests are carried out in silicone-coated tubes.

In this investigation 17 normal control subjects, 18 abnormal controls, and 35 patients with thrombosis were tested (Tables I and II). There were no significant differences in the mean clotting times between the three groups.

**Clotting Time of Capillary Blood in Glass Tubes (Dale and Laidlaw Method).**—This test was made on 66 normal subjects, on 22 abnormal controls, and on 33 thrombotic patients. The results are shown in Tables I and II. Again there was no significant difference between the mean results from the patients with thrombosis and from the control group. It will be seen that the use of capillary blood considerably shortens the clotting time, presumably because tissue thromboplastin is mixed with the blood as it is taken. There is therefore less scope for measuring a reduction in clotting time should this occur.

**Prothrombin Consumption Test.**—The results of this test on the "silicone" sera are shown in Table III. Again no significant differences were found between the three groups. The test was also made on the "glass" sera, to eliminate from the "silicone" data any sera which showed an abnormally low prothrombin consumption when the blood was allowed to clot in glass, because in these instances a pathological increase in the silicone prothrombin consumption might have been obscured. No such instances were found.

**Heparin Resistance Test.**—It has been shown by various workers that the blood of post-operative patients may "resist" the action of heparin (de Takáts, 1944; Waugh and Ruddick, 1944a, b). In other words, when comparable amounts of heparin are added to the blood of a normal person and a post-operative patient the clotting time may be more prolonged in the normal than in the patient's blood. If the blood of patients with thrombosis were unusually resistant to the action of heparin this might indicate an abnormally high thromboplastin activity. It was thought worth while to re-examine this test, using the technique of Waugh and Ruddick.

Both in normal people and in patients difficulty was encountered in using unaltered whole blood for the heparin resistance test. The order in which the tubes were filled and the efficiency with which the contents were mixed had an appreciable effect on the results. Thus the main experiment was carried out with citrated whole blood in which time was not such an important factor in setting up the experiment.

The results for the thrombotic patients are given in Table II. It was found that for normal people

the curves relating clotting time to heparin concentration varied greatly between persons, both when whole blood and when citrated whole blood were used (Fig. 1). The variation was so great that only a high degree of resistance could be con-

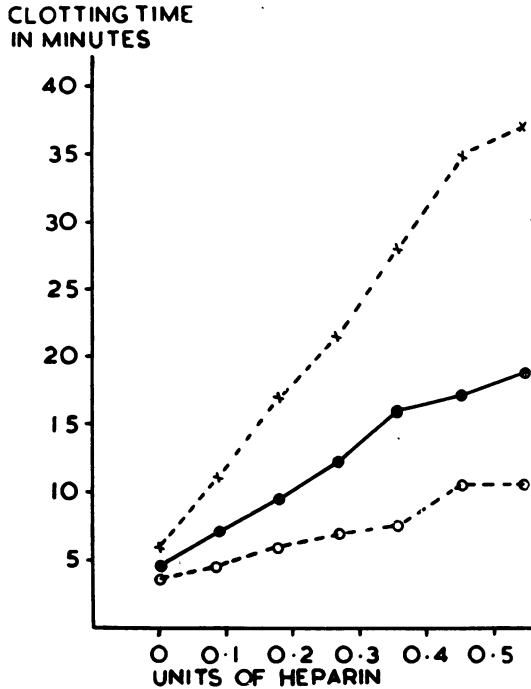


FIG. 1.—Mean and extreme ranges of clotting times for the Waugh-Ruddick test in 10 normal subjects, using citrated blood: one test was made on each subject. The heparin concentrations are calculated from the mean haematocrit of the group.

sidered significant of abnormality in an individual patient. It was thought that there might be some detectable difference between the control groups and the patients with thrombosis. The slope of the regression line of clotting time on heparin concentration was taken as a measure of resistance. A statistical analysis of the results showed no significant difference between the two groups, either using the data from the heparin tubes alone, or taking this in relation to the saline control tube clotting times. As the results expressed in either of these forms would be of limited interest, the values derived from the control groups have not been tabulated. Data were obtained from 30 normal controls (19 on citrated blood) and 31 abnormal controls (29 on citrated blood).

It has been shown by Carr and Fowler (1949) and by others that the heparin resistance test is

greatly influenced by the number of platelets present. This was confirmed in the present study by the thrombocytopenic patients, who gave significantly longer clotting times than other subjects in this test only. If the platelets are few, then the blood is more susceptible to the influence of heparin. From the work of Carr and Fowler it seems possible that the heparin resistance test, like the measurement of clot retraction, may be little more than a subsidiary unreliable method for assessing the relative number (or function) of the platelets.

### Discussion

None of the tests studied disclosed any significant difference in clotting function between patients with thrombosis and the control subjects, except that by the Lee-White method the whole blood clotting time in glass tubes was somewhat shorter in the healthy controls than in the other two broad groups. It must be realized that the tests used are crude tests which give widely varying results in normal people, and that, if more reliable tests could be evolved, measurable differences might be found between patients with and without thrombosis. The essential difficulty of the problem lies in the fact that the tests used must be tests of clotting function as a whole. Prothrombin and factor V (proaccelerin) are present in vast excess in normal plasma: even if an increase in either of these factors were demonstrated it would still have to be shown that this increase altered the clotting function. The limiting factor in normal coagulation is almost certainly the amount of available thromboplastin. Tests for this substance have not yet been used with the blood of patients with thrombosis.

In the present study patients with thrombosis and patients with a predisposition to thrombosis were compared with normal subjects and with bed patients with no thrombosis. No clinically important difference between the three groups was demonstrated. Nevertheless it could reasonably be claimed that the alteration in coagulability of the blood might be transitory and might take place just before the thrombosis occurred. In these circumstances patients with a fully developed thrombosis might not show any change in the coagulability of the blood.

If this is true then a test for a "prethrombotic state" would have to be carried out daily or even hourly if this state were to be demonstrated. None of the tests at present available could reasonably be carried out so frequently on large numbers of patients.

### Summary

The whole blood clotting time in glass and silicone-treated vessels, the prothrombin consumption in "silicone" serum, and the heparin resistance test on citrated blood have been investigated as tests for thrombosis.

Investigations were made on 82 normal subjects, 40 bed patients free of thrombosis, and 40 thrombotic patients. (Persons re-tested after an interval of several months, or in different clinical groups, were counted twice.)

Clinically important differences in test results could not even be demonstrated between the mean values for the three groups.

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