Coagulation tests on capillary blood

A screening procedure for use in small children

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SYNOPSIS A method of investigating the coagulation mechanism is described.

Results obtained by this procedure are compared with those of tests carried out on venous blood obtained simultaneously from normal subjects and patients with a variety of clotting defects. Good correlation was found between the results of P and P tests on venous and capillary blood, and also between thromboplastin screening tests on capillary blood and antihæmophilic and Christmas factor levels.

The methods described have proved reliable as a pre-operative screening procedure in routine use over a period of nearly two years.

Investigation of the coagulation mechanism is most reliably carried out on blood obtained rapidly from a clean venepuncture. In small infants and some older subjects, however, such specimens may be difficult to obtain; in such cases, free-flowing capillary blood is preferable to a venous sample obtained with difficulty through a small needle, in which contamination with tissue fluid and delay in taking are likely to result in early changes of coagulation before citrate is added. In this paper we describe a procedure by which a routine investigation of the blood coagulation mechanism can be carried out on 0.2 ml. of capillary blood; the results are compared with those obtained on venous blood taken from the same subjects simultaneously.

of samples from patients suspected of having a coagulation disorder, we have chosen to use a modification of the thromboplastin screening test of Hicks and Pitney (1957) as a test of blood thromboplastin formation, and the prothrombin and proconvertin (P and P) test of Owren and Aas (1951) as a test of the extrinsic coagulation system. This combination of tests will detect deficiencies of all known clotting factors except fibrinogen, as shown in Table I. As a simple test to exclude serious fibrinogen deficiency, the clotting time test of Dale and Laidlaw (1911) may be carried out on the capillary blood sample.

PRINCIPLES

Blood samples used for coagulation studies should be mixed with anticoagulant without delay in order to arrest the coagulation process as soon as possible. The most effective way of doing this in the case of capillary blood is to take a small measured quantity of blood directly into several times its own volume of diluting fluid containing sodium citrate; by this means, sufficient blood to provide nearly 2 ml. of diluted plasma can be obtained and the coagulation process halted within a matter of seconds. Such samples can be tested by any procedures requiring the use of dilute plasma. For the initial examination

TABLE I
RESULTS OF P AND P AND THROMBOPLASTIN SCREENING TESTS IN VARIOUS HAEMORRHAGIC STATES

Deficiency	P and P	Thromboplastin Generation Screening Test
Hageman factor Plasma thromboplastin antecedent Christmas factor (factor IX) Antihæmophilic factor (factor VIII) Factor V	} Normal	Abnormal
Factor VII		
Prothrombin		
Stuart-Prower factor (factor X) Combined deficiency of liver disease, vitamin K deficiency, etc.		
Fibrinogen		
	Abnormal	Normal
	Abnormal	Abnormal
	Normal	Normal

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If the screening procedures mentioned above reveal a defect, more specific tests may be carried out on the same sample; in this way, the exact nature of the clotting defect may be identified and its degree determined by means of quantitative assay procedures. This paper, however, is concerned with screening procedures only, and the details of specific assay methods will not be considered.

METHODS

SUBJECTS STUDIED Capillary blood and venous blood were collected as described below from normal adults and from patients of various ages with haemophilia, Christmas disease, or congenital deficiency of factor V, factor VII, or plasma thromboplastin antecedent (PTA). Blood was also taken from a series of adults on long-term anticoagulant therapy with phenindione (Dindevan) and from patients with acquired 'hypoprothrombinaemia' from various causes, particularly liver disease.

COLLECTION OF CAPILLARY BLOOD Anticoagulant-diluting fluid, 1.8 ml., of the following composition is measured into a capped polythene tube (60 × 15 mm.):

Trisodium citrate..... 2 g.
Sodium chloride..... 5.6 g.
Barbitone buffer, pH 7.35 (Owren, 1949)..... 200 ml.
Distilled water..... 800 ml.

Of this fluid 0.2 ml. is then drawn up from the polythene tube into a pipette calibrated to contain 0.2 and 0.4 ml. A well-warmed finger (or heel in the case of an infant) is stabbed with a sharp Hagedorn needle, and, after wiping away the first drop, blood is drawn up under the fluid in the pipette until the total volume is 0.4 ml. The whole is then washed out into the remainder of the fluid in the polythene tube, which thus contains 2.0 ml. of a 1 in 10 dilution of whole blood in buffered citrate-saline. Two heparinized microhaematocrit tubes are then filled from the same stab wound. The whole collection should not take more than one minute.

The dilute blood is centrifuged at 2,500 r.p.m. for 15 minutes, and the supernatant used in the tests described below. The subject's packed-cell volume (P.C.V.) is determined by the use of a microhaematocrit centrifuge, and used to calculate the actual dilution of the plasma tested (see below).

COLLECTION OF VENOUS BLOOD Nine parts of blood, obtained by clean venepuncture within a few minutes of taking the capillary sample, are mixed with one part of 3.1% trisodium citrate and centrifuged at 2,500 r.p.m. for 15 minutes.

PROTHROMBIN AND PROCONVERTIN (P AND P) TEST This was carried out by the method of Owren and Aas (1951) as modified by Biggs and Macfarlane (1957). Capillary plasma was tested after removal of the red cells by centrifugation as described above, but without further dilution. Citrated venous plasma was tested at a 1 in 10 dilution in the following solution:—

Trisodium citrate..... 1.5 g.

Sodium chloride..... 5.6 g.
Barbitone buffer, pH 7.3 (Owren, 1949)..... 200 ml.
Distilled water..... 800 ml.
The same final concentrations of citrate, NaCl, and buffer were thus obtained in the test dilutions of both venous and capillary plasma.

The clotting times in this system of the test plasmas were determined in duplicate in each case, and the mean clotting times converted to percentages of normal 'P and P' activity by interpolation on a standard dilution curve prepared from pooled normal venous plasma. In order that the results obtained on capillary and venous blood might be directly compared, the percentage activity of a 1 in 10 plasma dilution was calculated in each case by correcting the observed activity for the diluting effect of the anticoagulant, which varies with the P.C.V.; the calculations involved may be reduced to the following formulae:

$$\begin{aligned} \text{I Capillary P and P (\%)} &= \frac{P_c \times 100}{(100 - \text{P.C.V.})} \\ \text{II Venous P and P (\%)} &= \frac{P_v (100 - 0.9 \text{ P.C.V.})}{90 - 0.9 \text{ P.C.V.}} \end{aligned}$$

where P_c and P_v are the P and P percentage activities respectively of the capillary and venous plasma dilutions tested. In practice, it is always necessary to correct capillary plasma results in this way, as differences in P.C.V. have a significant effect on the final plasma dilution. Similar differences have little effect on the final dilution of venous plasma, as the actual 1 in 10 dilution is carried out in this case on citrated plasma and not on whole blood; the correction factor is only applied here in order that the venous and capillary results may be directly comparable, and is unnecessary for the interpretation of venous results as such.

THROMBOPLASTIN SCREENING TEST In most instances, the method of Hicks and Pitney (1957) was used, with Inosithin¹ (0.02 g. per 100 ml.) as the source of phospholipid; capillary plasma was tested after removal of the red cells without further dilution. During the period of this study, it was found that greater reproducibility of the results of this test could be achieved by adding an optimum amount of kaolin to the mixture of dilute plasma and Inosithin, and incubating at 37°C. for five minutes before the addition of calcium chloride. Some of the subjects seen towards the end of the study were tested by this modification of the standard procedure.

As the results of this test are not capable of expression in an accurate quantitative manner, no attempt was made to compare results obtained on capillary blood directly with those obtained on venous samples from the same subject, as in the case of the P and P test. Capillary blood from at least one normal control subject was tested in parallel with each sample obtained from a patient, and the second-stage clotting times compared directly.

ANTIHAEMOPHILIC FACTOR ASSAYS Four of the 16 haemophilic patients were infants from whom venous blood could not easily be obtained; in these cases, antihæmophilic factor assays were carried out on capillary blood

¹Associated Concentrates, Woodside, L.I., N.Y.

In all the other cases of haemophilia investigated, anti-haemophilic factor assays were performed on venous samples by a modification of the thromboplastin generation test similar to that described by Pitney (1956), in which the ability of the patient's Al(OH)_3 -adsorbed plasma to correct the defect of Al(OH)_3 -adsorbed plasma from a severe haemophiliac was compared with that of Al(OH)_3 -adsorbed pooled normal plasma.

CHRISTMAS FACTOR ASSAYS In each of the seven patients with Christmas disease studied, the diagnosis was based on the result of a thromboplastin generation test, and the level of Christmas factor was determined by a one-stage method in which the correction of the kaolin clotting time of Christmas-factor-deficient plasma by serial dilutions of patient's plasma was measured.

RESULTS

EFFECT OF DELAY IN COLLECTING CAPILLARY BLOOD

It was thought that a likely source of error in results obtained in capillary blood would be delay in the collection of blood after pricking the finger; this might allow some of the early changes of coagulation to occur at the site of injury before the sample was obtained. In order to determine the importance of speed of collection, three separate capillary blood samples were taken consecutively from the same fingerprick in a small series of subjects, and thromboplastin screening tests and 'P and P' determinations were carried out in parallel on all three specimens. The time taken to collect each specimen varied between 20 and 60 seconds, and the whole collection was completed in each case within three minutes.

It was found that there was no significant difference in 'P and P' results between any of the three specimens nor between the first two specimens in the results of the thromboplastin screening test; in this test, however, the clotting times obtained with

the third specimen were often significantly longer than those with the first two. Typical sets of results are shown in Table II. These findings indicate the importance of rapid collection of capillary blood samples, and suggest that specimens collected within one minute will give reliable results in practice.

PROTHROMBIN AND PROCONVERTIN (P AND P) TEST

The P and P test was performed on samples of venous and capillary plasma obtained simultaneously from 21 normal subjects, 40 patients receiving anti-coagulant treatment with phenindione, and 10 patients with various congenital clotting defects. The results, corrected for dilution, are plotted in Fig. 1, from which it can be seen that there is good correlation between the capillary and venous results, particularly in the 0 to 60% range.

FIG. 1. Correlation between 'P and P' results obtained on venous and capillary blood samples. The diagonal line represents 100% correlation. The coefficient of correlation between the two sets of readings is 0.969.

TABLE II

RESULTS OF TESTS ON THREE CONSECUTIVE CAPILLARY BLOOD SAMPLES FROM SAME FINGERPRICK

Subject	Blood Sample	Thromboplastin Screening Test				P and P (%)
		Incubation Time (min.)				
		2	4	6	8	
		Clotting Time (sec.)				
A	I	22.5	12	15.5	20.5	89
	II	80	12	13.5	17	85
	III	105	29	15	19	85
B	I	71	13.5	15	16	82
	II	48	14	15	—	78
	III	41	15	15.5	—	82

THROMBOPLASTIN SCREENING TEST Thromboplastin screening tests were carried out on a total of 29 capillary blood samples from patients with haemophilia and Christmas disease of various degrees of clinical severity, in whom antihaemophilic factor or Christmas factor assays were also performed. In eight instances, the antihaemophilic factor or Christmas factor had been raised to values between 15 and 50% by replacement therapy. In Fig. 2, the shortest second-stage clotting times obtained in the thromboplastin screening test are plotted against the antihaemophilic factor and Christmas factor levels;

FIG. 2. The relationship between the minimal second-stage clotting time in the thromboplastin screening test performed on capillary plasma and the level of antihemophilic or Christmas factor. ●, haemophilia (antihemophilic factor assay on venous plasma); ○, haemophilia (antihemophilic factor assay on capillary plasma); ×, Christmas disease. All the normal controls gave minimal clotting times between 10 and 14 seconds in the thromboplastin screening test.

the screening test times were outside the normal control range in every case in which the antihemophilic factor or Christmas factor was below 35%, and tended to be longest in the cases with the lowest levels of antihemophilic factor or Christmas factor.

In Figs. 3 to 5 are shown the results of 'P and P' and thromboplastin screening tests carried out on capillary blood samples from patients with 'hypoprothrombinaemia' resulting from vitamin K deficiency and with congenital deficiencies of factor V and factor VII respectively. These examples illustrate the three patterns of abnormality detectable by the use of these two tests (cf. Table I).

Many children have been investigated by these methods before operation, on account of a history suggestive of a bleeding tendency. Whenever the shortest clotting time in the thromboplastin screening test exceeded that of the normal control by three seconds or more, or the 'P and P' level was below 50%, further investigation of the coagulation mechanism was carried out. All other patients were regarded as suitable for operation provided that the platelet count and bleeding time were also normal, and none of these bled excessively.

FIG. 3. Thromboplastin screening test on capillary plasma from a patient with vitamin K deficiency.

FIG. 4. Thromboplastin screening test on capillary plasma from a patient with congenital factor V deficiency.

FIG. 5. Thromboplastin screening test on capillary plasma from a patient with congenital factor VII deficiency.

DISCUSSION

The main theoretical objections to the use of capillary blood for tests of the coagulation mechanism concern the difficulty of obtaining a large enough volume of blood before the early changes of coagulation have occurred; this difficulty is aggravated by the likelihood of contamination of the sample with tissue fluid. The method described here, in which a very small volume of blood is taken directly into anticoagulant, largely overcomes this obstacle by ensuring rapid collection; the reliability of the method is shown by the fact that two consecutive samples could always be obtained in this way without significant change in clotting activity.

Somewhat similar methods of collection have been used by other workers to obtain blood for prothrombin time estimations (Dyggve and Lund 1954; Miale and Winningham, 1960), but those who have attempted more extensive studies of the coagulation process on capillary blood have usually taken larger volumes. Ashley (1958), for example, took 0.9 ml. of capillary blood into 0.1 ml. citrate from a small series of normal subjects and obtained results in a battery of coagulation tests which were comparable with those of similar tests on venous blood from the same subjects; he showed no direct evidence, however, of the reliability of his procedure as a means of detecting coagulation defects.

The methods described here have proved their value in practice, as they have been used with complete success over a period of nearly two years as a pre-operative screening procedure in children suspected of having a bleeding tendency. During this period, seven cases of haemophilia, two of Christmas disease, and one of PTA deficiency have first been detected by these methods. That abnormal results were obtained in all cases in which the level of antihæmophilic or Christmas factor was known to be below 35% indicates that degrees of deficiency of these factors likely to be associated with excessive bleeding at operation would not be missed by the use of these tests on capillary blood.

One of the chief objects of screening tests of

coagulation is to anticipate the need for special measures when surgery is indicated, and it must be emphasized that the results of such tests, whether they be carried out on capillary or venous blood, should always be interpreted in the light of a careful clinical history. Whenever abnormal results are obtained, or clinical considerations suggest that near-normal results are not to be relied upon, additional, more specific tests should be carried out in order to reach an exact diagnosis. Semiquantitative tests for individual clotting factors may be performed on the same capillary sample by determining its ability to correct plasmas with known specific deficiencies in appropriate test systems, and in most instances the diagnosis can be made in this way. This is not to suggest, however, that results obtained on capillary blood are as reliable as those on blood obtained from a clean venepuncture; if they are readily obtainable, venous samples should always be used for detailed coagulation studies, especially in the assessment of mild disorders.

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