A suggested schedule for the rapid investigation of acute haemostatic failure

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SYNOPSIS

A small group of tests is proposed for the rapid investigation of acute bleeding states. These techniques are within the compass of many clinical laboratories, and the whole scheme may be run through in one to one-and-a-half hours.

Acute bleeding states may arise in association with concealed accidental haemorrhage and other obstetrical complications, surgery of the lung, carcinomatosis, and other conditions. It is characteristic for multiple haemostatic defects to be produced, the pattern varying from case to case. If the haematologist is to contribute to clinical management he must be able quickly to identify the cause of bleeding so that he may be able to advise promptly on the appropriate replacement therapy or on the risks of surgery.

The following scheme of investigation is therefore offered for a rapid appraisal of those facets of haemostasis which usually seem to be affected. A fuller discussion has been given by Ingram, Norris, and Tanner (1960) with an illustrative obstetrical case history; an example from chest surgery has been described by Ingram and Mann (1959).

The two commonest processes underlying acute failures of haemostasis appear to be defibrination and fibrinolysis; it has been thought that either may be initiated by an escape of tissue material into the blood stream. More rarely, a heparin-like inhibitor may appear in the blood.

Defibrination and fibrinolysis may occur separately or together. Their effects are similar, for they both characteristically produce thrombocytopenia, fibrinogenopenia, and deficiencies of antihaemophilic globulin (AHG) and factor V. The following scheme is therefore designed to test these four components, as well as to detect fibrinolysis and heparinaemia. The other types of inhibitors which have been described do not appear to develop so acutely (with the possible exceptions of one associated with fibrinolysis described by Niewiarowski, Kowalski, and Stachurska, 1959, and of the effect discussed by von Kaulla and Swan, 1958).

A few notes on treatment are appended.

METHODS

All tests except the platelet count are made on citrated plasma (9 volumes of blood added to 1 volume 3·8% sodium citrate). The following five tests are used: (1) Platelet count; (2) Quick's prothrombin time for activity of factor V (a long prothrombin time due to factor V deficiency would be shortened by the addition of Al(OH)₃-treated normal plasma 1 in 5 or 1 in 10); (3) rapid fibrinogen determination; (4) a simple modification of the thromboplastin generation screening test of Hicks and Pitney (1957) for antihaemophilic globulin activity (although also affected by factor V); (5) the thrombin clotting time, providing a fibrinogenolysis test (adapted from Wilhelm, Miles, and MacKay, 1955), and a simple test for heparinaemia. Tests (1) and (2) are, of course, done by the usual methods. Tests (3), (4), and (5) are described below.

TEST 3

This is the determination of the weight of fibrin obtained on clotting a measured volume of plasma (Ingram, 1952a), omitting drying the clot in hot air.

Method

Equal volumes of plasma and M/40 CaCl₂ (say 2·0 ml) are mixed and incubated for about 10 minutes, a wooden applicator stick being placed in the tube. When a firm clot has formed this is wound on to the stick, expressing the serum. The stick is then transferred to a tube of water and the serum returned to incubate in case further clot forms, which is harvested as before. The total clot is then peeled off the stick (like stripping the rubber grip off the handle of a cricket bat) and pressed between filter papers, and then transferred to acetone, to dry and harden, for exactly 10 minutes. After decanting, the clot is transferred to the warm hand, and the remaining acetone rapidly evaporated by gentle blowing; the clot is then immediately weighed to the nearest 0·2 mg.
The clot may of course be determined by other methods if preferred, but gravimetry has been found very convenient for a 24-hour service, since a chemical balance requires no ‘warming up’ and most laboratory workers are familiar with its use.

Weight of clot

\[
\frac{\text{concentration of fibrinogen in}}{\text{Volume of plasma in}} = \text{mg./100 ml. (the conventional unit)}
\]

e.g., \(\frac{21 \text{ mg.}}{7-3 \text{ ml.}} = 0.35 \text{g./100 ml.}\)

Errors

The true value will be overestimated by the water remaining in the clot, and underestimated by the dilution of the plasma with the citrate. These errors may be corrected as follows: (a) For retained water, reduce the observed weight of the clot by subtracting 15% plus an additional \(\frac{4}{5}\)% per mg. observed weight. (This correction was determined empirically by observing the further loss in weight which occurred after heating clots in hot air as described in the original method.) (b) For citrate dilution, multiply the value of the concentration of fibrinogen (as amended by \(a\) above) per millilitre of plasma by

\[
\text{Percentage plasma in citrated blood}
\]

\[
\text{Percentage plasma minus percentage citrate}
\]

(Since the proportion of citrate in the whole citrated blood is known, the correction may be calculated from the haematocrit of this blood.) Note that these corrections tend to cancel each other, and that in fibrinogenopenia, when a very small clot is obtained, they have a small effect anyway and so for clinical purposes they may often be ignored.

The following is an example when the haematocrit of the citrated blood is 34%.

\[
100 - 34 = 66
\]

\[
\frac{1}{5} \times 7.3 = 4%, \quad 15% + 4% = 19% \quad 19% \text{ of } 7.3 = 1.4 \text{ mg.}; \quad 7.3 - 1.4 = 5.9 \text{ mg.}
\]

corrected value = \(\frac{5.9 \text{ mg.}}{2.2 \text{ ml.}} = 0.32 \text{g./100 ml.}\)

To estimate a patient’s plasma fibrinogen deficit, reckon the blood volume as \(1/10\) body weight; calculate the plasma volume from this estimate and the venous haematocrit; multiply the value obtained by the observed fibrinogen concentration and this gives an estimate of the total plasma fibrinogen. Similarly calculate the total plasma fibrinogen had the concentration been normal (say 0.3 g./100 ml.). The difference between these two calculated values estimates the plasma fibrinogen deficit.

### Suggested Schedule for the Rapid Investigation of Acute Haeostatic Failure

**TABLE I**

**SIMPLIFIED THROMBOPLASTIN GENERATION SCREENING TEST**

<table>
<thead>
<tr>
<th>Time (min.)</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Set up tube 1 (normal plasma)</td>
</tr>
<tr>
<td>1</td>
<td>Set up tube 2 (patient’s plasma)</td>
</tr>
<tr>
<td>2</td>
<td>Set up tube 3 (50 : 50 mixture of plasmas)</td>
</tr>
<tr>
<td>3</td>
<td>3 min. sample from tube 1</td>
</tr>
<tr>
<td>4</td>
<td>3 min. sample from tube 2</td>
</tr>
<tr>
<td>5</td>
<td>3 min. sample from tube 3</td>
</tr>
<tr>
<td>6</td>
<td>6 min. sample from tube 1</td>
</tr>
<tr>
<td>7</td>
<td>6 min. sample from tube 2</td>
</tr>
<tr>
<td>8</td>
<td>6 min. sample from tube 3</td>
</tr>
</tbody>
</table>

Each tube is set up with 0-1 ml. of each of the appropriate plasma 1 in 10, platelet substitute, and M/40 CaCl₂, and at the times indicated is sampled onto 0-1 ml. of normal undiluted citrated plasma to which has just been added 0-1 ml. M/40 CaCl₂.

To incubate, with a pipette, in a third tube. At the next minute transfer 0-1 ml. from the first tube to 0-1 ml. of normal citrated plasma to which 0-1 ml. of M/40 CaCl₂ has just been added (5 to 10 sec. before) and measure the clotting time. At the next minute perform this procedure on the second tube and at the next minute for the third tube. At the following three minutes repeat the procedure again for the first, second, and third tubes in that order.

It is convenient to arrange the three reaction tubes and six clotting time tubes (containing normal plasma) in the water bath before starting the test. It will be apparent that this procedure yields a three-minute and a six-minute sample by the Hicks and Pitney procedure for normal plasma, the patient’s plasma, and a mixture of the two so that in one run it becomes apparent whether the patient’s plasma behaves abnormally in this test and if so whether this is due to a deficiency (when the results from the third tube will approximate to those of the first) or to an inhibitor (in which case the results of the third tube will approximate to those of the second). For confirmation the whole procedure may be repeated in the reverse order, i.e., 50 : 50 mixture, patient’s plasma, control plasma, thus obtaining two replicate readings at each sampling time from each tube. The reversal of the order represents an attempt to eliminate a trend due to the passage of time (Ingram, 1955).

**ALTERNATIVE PROCEDURE CONTROLLING ‘CONTACT’**

The above test is conveniently rapid and adequate to detect gross defects but it ignores differences in the degree of ‘contact’ to which the plasmas have been subjected. Variations between the ‘contacting’ of the samples may lead to variations in the rate of their reactivity, and may thus blur the differences between normal and mildly abnormal plasmas.

It is difficult to ensure that contact is equally prevented in all samples but an equal, vigorous contacting can be applied by a brief preincubation with kaolin (Margolis, 1957, 1958). This provides a better technique, although slight inconveniences introduced by this procedure are that an additional reagent is required, and that though its action, thromboplastin generation becomes so rapid that is more informative to sample at 2 min. and 4 min. instead of at 3 min. and 6 min.; hence, in the ‘running’
design, only two, and not three, reactions can be handled concurrently. The 50 : 50 mixture of patient's and control plasma must therefore be tested separately. (The design thus allows this mixture to be paired with a test on 1 in 20 control plasma for a comparison with the effect of simple dilution if desired.) Note that by 2 min. the reaction mixture containing control plasma may well have reached maximum activity so that it may in fact be simpler, for occasional use, not to employ the running design, but to test each plasma separately in the traditional manner and to sample at 1, 2, 3, and 4 min. after completing the reaction mixture, having preincubated the kaolin and the plasma for two minutes. A running design can then be used to decide between deficiency and an inhibitor, and to test the effects of various additions, as described immediately below.

Method A convenient stock suspension contains light kaolin, 10 mg./ml.; this is diluted 1 in 10 for use. Place 0·05 ml. of the working suspension in a tube and add 0·1 ml. of control plasma in 10; mix and put to incubate. For the running design, one minute later make a corresponding mixture with patient's plasma in 10 and put to incubate. At the next minute, add platelet substitute and M/40 CaCl₂ to the control tube, as in the previous method; and at the following minute similarly complete the reaction mixture for the patient's plasma. At the subsequent four successive minutes subsample the control reaction, the patient's reaction, and again the control reaction and the patient's reaction, according to the previous method. Each plasma will then have received two minutes' preincubation with kaolin and will have yielded samples at two and four minutes after the completion of the reaction mixtures. The principles of interpretation are of course on the same lines as in the previous method.

If the test suggests that the patient's plasma is deficient rather than inhibitory do another run in which the control plasma only has been adsorbed with alumina (Biggs and Macfarlane, 1957, pp. 387, 388) before dilution. This should of course give an abnormal run when tested alone, but if in the 50 : 50 mixture it corrects the patient's plasma about as well as did the untreated normal plasma, then the patient's blood must be deficient in either antihaemophilic factor or factor V; and if replacement infusion is indicated, fresh normal acid-citrate-dextrose (A.C.D.) plasma should be given. If adsorbed normal plasma does not correct but if, on the other hand, aged normal serum 1 in 10 corrects the patient's plasma about as well as did the untreated normal plasma in the original test then the patient may be treated with A.C.D. plasma which has been stored for up to one month at -20°C, since the 'serum' factors appear to be stable in this material (Brafield and Case, 1956).

PLATELET SUBSTITUTES Platelet suspensions are tedious to prepare and do not keep well but several substitutes are available. A preparation from brain was described by Bell and Alton (1954); a soy-bean phospholipid, Inosithin, may also be used, at about 0·02 g./%


TEST 5 Fibrinolysin acts in the body as a fibrinogenolyis, and this is also the basis of the test described by Wilhelm et al. (1955). Separate samples of the patient's and the control plasmas, e.g., 2·0 ml., are incubated concurrently, and at intervals 0·1 ml. samples are withdrawn and the thrombin clotting times measured. If the fibrinogen is being gradually destroyed in the patient's incubated sample the thrombin clotting times on this plasma will progressively lengthen.

Thrombin clotting time test Human thrombin is diluted with M/80 CaCl₂ so that when 0·2 ml. is added to 0·1 ml. of normal citrated plasma the clotting time is approximately 10 sec. This test is also sensitive to the presence of heparin so that if readings are obtained from the two plasmas immediately they are put to incubate and the patient's plasma gives a longer clotting time ab initio, this might be due to a heparin-like anticoagulant or to a pre-existing reduction in the fibrinogen concentration of the plasma. If the clotting time is prolonged by heparin, the difference between the patient's and the control times will be reduced by adding to each plasma a volume of toluidine blue solution in 1,000 to 1,000,000 between repeating the test; if the long clotting time is due to hypofibrinogenema, it will similarly be shortened by adding a volume of fibrinogen solution, approximately 200 mg. per 100 ml. Heparinemia is, of course, rare, and once this has been excluded, proceed with the fibrinogenolysis test by measuring the thrombin clotting time on further 0·1 ml. subsamples from each plasma at intervals of, say five, 10, 20, 40, and 60 minutes according to the rate of change observed. If the fibrinogen concentration is already low in the patient's sample, fibrinogen must be added to both incubated plasmas in order to work this test, but either way, if no consistent difference between the plasmas is apparent after two hours' incubation, the test is negative for clinical purposes. Note that the control clotting time may also lengthen somewhat as the test proceeds, but this is immaterial, since the significant finding is an increasing difference (conveniently expressed as a proportion) between the clotting times from the two plasmas.

SETTING UP THE TESTS It will be apparent that these tests may be run concurrently and the following plan is suggested:

1 Obtain 10 ml. citrated blood from the patient and from a control subject; (2) set up the haematocrit of the patient's citrated blood, and also (3) the patient's platelet count, either on the venous or a capillary blood sample according to local practice. (4) Spin both the citrated bloods (say, 3,000 r.p.m. for 10 min.) to obtain citrated plasma. (5) Set up the fibrinogenolysis test and (6) take the initial readings to exclude haezinemia; (7) set up the fibrinogen estimation and (8) take the next pair of readings from the fibrinogenolysis test; (9) do Quick's prothrombin time in the two plasmas; (10) repeat the fibrinogenolysis test; (11) examine the fibrinogen estimation tube and harvest the clot if it seems ready; (12) prepare the reagents for the Hicks and Pitney test and (13) repeat the fibrinogenolysis test if the rate of change in previous readings warrants it. It then only remains (14) to count the platelets, (15) perform the Hicks
and Pitney test, (16) weigh the clot, and (17) repeat the fibrinogenolysis test in the most convenient order. The whole procedure takes an hour to an hour and a half, single handed.

Note that the following reagents may be maintained at −20°C, from which dilutions may be prepared for immediate use: (1) Human thrombin, approximately 50 units per ml., and (2) human fibrinogen, 1 g. per 100 ml.

It has been found that the inclusion of a quarter-volume of the veronal buffer of Owren (1949) seems to prevent denaturation on repeated freezing and thawing. (3) The platelet substitute as a colloidal solution of Inosithin, 1 g. per 100 ml., or Bell and Alton’s (1954) brain extract as described by the authors.

DISCUSSION

These tests are intended for laboratory use under the supervision of the clinical pathologist and are not bedside tests (in the sense of tests to be performed by clinicians) although they are simple enough to be done in a side-room equipped with a centrifuge, water bath, and the basic glassware. There is thus no mention of tests made on whole blood, such as the clotting time or the clot observation test, which are less efficient than the tests described in this paper and are only worth carrying out when laboratory facilities are not available.

The thrombin titre test of Sharp, Howie, Biggs, and Methuen (1958) is not mentioned because it is thought that the same information will be given by the test for fibrinogenolysis described above and that this test is more convenient to work in with other procedures which are proposed.

For the detection of a deficiency of AHG in the acquired bleeding states, the two-stage test of Hicks and Pitney is preferred to one-stage tests, e.g., of the type proposed by Margolis (1958), because in the circumstances under discussion here, the one-stage tests might give abnormal results due to a deficiency of fibrinogen or even because prothrombin had become seriously depleted. These tests would therefore be difficult to interpret in terms of the early stages of blood coagulation which involve AHG. In the two-stage test, only the early phases of coagulation depend on materials supplied by the patient’s plasma so that interpretation should be easier.

FIBRINOGEN ESTIMATION Numerous techniques for the estimation of plasma fibrinogen concentration are of course available and other methods than that described may be preferred by various workers. It is, however, thought important to estimate what coagulates rather than what precipitates at a defined salt concentration or under other unphysiological physical conditions, and the described method has been found convenient and rapid for emergency use.

The normal ranges of clotting tests are difficult to define briefly. Normal adult values for the non-pregnant state are given or discussed by Ingram (1952b) for fibrinogen concentration, by Hicks and Pitney (1957) for the thromboplastin generation screening test, and by Ingram and Armitage (1959) for Quick’s prothrombin time; published normal values for late pregnancy are discussed and a personal series is given by Ingram et al. (1960). In particular, the last paper gives the basis for choosing 3 min. and 6 min. sampling times in the Hicks and Pitney test.

INDICATIONS FOR TREATMENT There are two principles in treating acute haemostatic failure. Fundamentally, the patient’s clinical condition must be resolved or limited, and if this can be achieved the patient’s haemostatic mechanisms will return to normal. Meanwhile, as a holding operation, the haemostatic defects should be kept above their critical levels by infusing the appropriate blood derivatives (fibrinogen, plasma, etc.). It is to obtain evidence on which to base the choice of the appropriate infusion that the above tests are designed. The clearest indication for infusing a particular component found to be abnormally low in the patient’s blood is to show by successive tests that the deficiency is progressively worsening, but clearly it is usually necessary in practice to decide on the results of single estimates. It may therefore be useful to suggest critical levels below which infusion should be considered. Table II gives working values for the platelet count, the fibrinogen estimation, and the thromboplastin generation screening test.

A potent and apparently safe antifibrinolytic drug is now available. Nilsson, Sjoerdsma, and Waldenström (1960) gave ε-amino caproic acid (εACA) in doses of up to 6 g. intravenously, and by mouth to a daily total of 36 g., with evidence of antifibrinolytic effect in vivo, to patients showing marked fibrinolysis. Where fibrinolysis has gravely depleted haemostatic factors these deficiencies should presumably also be made good as suggested above.

After treatment with blood or blood derivatives, the tests found initially abnormal may be repeated to check progress. In particular, if purified fibrinogen has been given, the fibrinogen estimation may be repeated at the end of the infusion and again after an interval of some hours. If the reading immediately after the infusion is lower than would have been
indicates the multiplication factors by which the subsample clotting times from the patient’s plasma reaction mixture are prolonged over those from the control plasma reaction mixture at the sampling times indicated. These critical values correspond to about 30% of the normal A.H.G. activity, with factor V normal (assume this if Quick’s prothrombin time is normal).

expected from a consideration of the dose given and of the patient’s blood volume, and if the subsequent reading is still lower, it may be concluded that the fibrinogen-consuming process is still active in vivo.

relative value of the tests The essence of this communication is that in the acute bleeding states, multiple haemostatic defects may be found, and that for the analysis of these conditions the pathologist therefore requires a group of tests which, between them, will detect the various abnormalities which have been encountered.

Nevertheless it may not always be possible to make all the tests proposed; and for these occasions it is suggested that the three most important would be the platelet count, the fibrinogen estimation or the thrombin clotting time, and the thromboplastin generation screening test. If all these are normal, an important defibrination or fibrinolytic process can be excluded; if, however, these first are abnormal, the other techniques should be made to identify the type of abnormality which is present.

I am indebted to Dr. R. M. Hardisty, of the Hospital for Sick Children, Great Ormond Street, for introducing me to Inosithin, and also for a discussion of the use of kaolin in Hicks and Pitney’s test, and to Mr. A. I. S. Macpherson, of the Royal Infirmary, Edinburgh, and to Dr. G. Wetherley-Mein, of St. Thomas’s Hospital, for discussions of the surgical risks in thrombocytopenia and of platelet transfusion.

REFERENCES