Technical methods

An apparatus for measuring the tensile strength of blood clots

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SYNOPSIS An apparatus is described which uses the principle of the ballistic pendulum to measure the tensile strength of blood clots formed in special cuvettes. The method appears to have a reproducibility which would allow a study to be made of the factors influencing clot strength. A brief survey of the findings with normal blood and with blood from cases of thrombosis and haemophilia shows significant differences between the mean values for each group.

The function of blood clots in haemostasis and repair must depend to some extent on their adhesiveness, tensile strength, and elasticity, and it would be of interest to know if variations in these properties are related to abnormal bleeding, thrombosis or delayed healing. Elasticity has been studied by Hartert (1948) and others, using the thromboelastograph, and by Scott Blair and Burnett (1960). Lalic and Copley (1942) estimated 'clot firmness' by measuring the pressure required to force clots through a constricted tube. Morrison and Doppelt (1954) have measured the adhesion of clots to different surfaces.

The measurement of the tensile strength of a whole blood clot is complicated by the fact that it is so friable that the mechanical anchorage to which traction is applied is likely to cause tearing at the site of attachment. In consequence, most previous work has been limited to plasma or fibrinogen clots, which are much tougher than those formed by whole blood. These have been studied by Tarlov, Goldfarb, and Benjamin (1942) who used rubber-covered clamps to grip the clot, and attached weights to its free end, and by Neurath, Dees, and Fox (1943) who incorporated gauze in the clot during its formation and used these points of attachment for suspension and loading.

The apparatus described here was designed to measure the tensile strength of whole blood clots without any previous manipulations. It is applicable to any other coagulable fluid, and can be modified to measure adhesion to a given surface. In measuring tensile strength, however, adhesion of the clot to its container is not a requirement. A brief description of the apparatus is contained in the proceedings of a symposium on the flow properties of blood (Macfarlane, Tomlinson, and Excell, 1960).

Received for publication 30 November 1960

PRINCIPLE

Blood taken by venepuncture is allowed to clot in a special cuvette which has a dumb-bell shaped cavity. The complete cuvette is assembled from two identical halves, the opposing faces of which are flat and fit accurately, the junction between them passing through the mid-point of the narrow, cylindrical 'neck' which connects the two wide cavities of the dumb-bell (Fig. 1). When clotting has occurred, the two half cuvettes are mechanically united by the clot, since its two expanded portions cannot pass through the narrow neck, and traction applied to one half cuvette will be transmitted to the second by the 'isthmus' of clot until it breaks.

The principle of the ballistic pendulum is used to measure the force applied to the clot. One half cuvette is located in a carrier at the end of a light but rigid pendulum (the measuring pendulum) hanging vertically from a bearing. The other half cuvette is located in a similar carrier, also attached to a pendulum arm (the traction pendulum) which swings from the same bearing as the first but is free to move independently of it. The two carriers are adjusted to be in register, so that when the cuvette is put into them the two half cuvettes remain aligned and in contact. The two pendulums are now connected only by the clot which connects the two half cuvettes. An electric winch moves the traction pendulum round the arc of a circle, and the measuring pendulum follows it, drawn by the tension in the clot. The fact that both pendulums have a common axis ensures that the two half cuvettes maintain their alignment until separation occurs. The force necessary to maintain the measuring pendulum at an angle, θ , to the vertical is directly proportional to $\sin \theta$, and since this force is transmitted by the clot, the tension in the clot increases as the movement progresses. When the tension is great enough the clot breaks, and the maximum deflection reached by the measuring pendulum is a measure of the maximum tension achieved. This deflection is recorded by the simple method of arranging for a sprag attached to the measuring pendulum to trail over a surface below it, offering little resistance to the forward movement of the pendulum, but preventing any reverse movement. Thus, as soon as the clot breaks, the measuring pendulum becomes stationary at maximum deflection, its position being indicated on a scale.

Since the friction of the bearings and sprag is negligible the instrument can be calibrated from a knowledge of the dimensions and weight of the measuring pendulum.

CONSTRUCTION

The cuvettes are machined from perspex rod to the sizes indicated in Fig. 1. Perspex coverslips are also provided. The carriers of the pendulum are also machined from perspex, with semicircular grooves to accommodate the flanges of the cuvette. When in the starting position

Section A - A

FIG. 1. Section and plan of the perspex cuvette (actual size).

FIG. 2. Side and end elevations of the cuvette carrier (actual size) showing the separating screw (marked x) and hinged sprag (y).

the two carriers are so aligned that they form a trough-shaped receptacle into which the cuvette fits accurately. To provide the small, carefully controlled separation of the half cuvettes necessary to break the vaseline seal (see below) a screw in the carrier of the traction pendulum bears on the carrier of the measuring pendulum (Fig. 2).

The traction pendulum is built of brass strip, since its weight is not a consideration, but the measuring pendulum has to be as light as possible, in order to ensure reasonable sensitivity, and sufficiently rigid to prevent lateral displacement. This pendulum consists of a fibre-glass tube, 4 cm. in diameter, with walls 1 mm. thick, and complete with carrier and a half cuvette filled with blood clot, weighs 200 g. Both pendulums are 1 metre long. The traction pendulum is suspended from plain bearings and the measuring pendulum from a needle and cup bearing arranged concentrically, both being supported on a braced upright constructed of Dexion angle strip (see Fig. 3), mounted on a base board, which can be levelled by means of screw legs. Two adjustable stops prevent the measuring pendulum from moving left-handed away

from its position of equilibrium. A pointer on the measuring pendulum travels over a scale directly calibrated in grams of tension. Behind the scale is a strip of metal, following the curvature of the arc, and covered with 'elastoplast', forming the surface over which the sprag trails, and into which it digs at the first reversal of movement. The sprag itself is a small steel needle, hinged to the lower end of the measuring pendulum. The sprag can be 'tripped' by the finger to re-set the pendulum to zero after a reading has been taken (Fig. 4).

The cuvette is moved at a constant speed of about 1.5 cm./sec. by an electric winch which can be reversed in order to return the pendulum to its starting point.

OPERATION

The cuvette is carefully washed and dried. After very lightly greasing the faces with vaseline, the half cuvettes are assembled, care being taken to prevent any grease exuding into the bore when the two halves are pressed together. A coverslip is applied, after greasing, to the

FIG. 3. The complete tensiometer showing the two pendulums with half-cuvettes in position on the carriers. In this illustration the clot has broken so that the measuring pendulum has come to rest and the traction pendulum has moved away from it.

lower end and the assembled cuvette is mounted in a holder which maintains the halves in alignment, and allows filling through the open top. Blood is drawn by syringe, and the cuvette is filled by inserting the needle to the bottom, so that air locks and bubbles are avoided, and expelling blood until it is level with the top; the volume of blood required is about 2.5 ml. The filled cuvette is then incubated at the required temperature, the onset of coagulation noted by tilting, and at the required time after clotting, testing is carried out. A cuvette is placed horizontally in the pendulum carriers shown in figure 2. The vaseline seal is then broken by carefully turning the screw, x, until the half cuvettes are separated by about 0.5 mm., no significant stress being applied to the clot by this movement. Once the seal is broken, the isthmus of the clot is the only physical connexion between one carrier and the other, and traction is applied by switching on the electric winch. At first the two pendulums move together; the clot begins to extend, it breaks, and the measuring pendulum remains at its highest point. The scale reading is recorded, the cuvette halves are removed, the measuring pendulum is returned to zero by releasing the sprag, the traction pendulum by reversing the motor, and the operation can be repeated

FIG. 4. Close-up view showing the lower ends of the two pendulums with half-cuvettes located in their carriers. The pointer attached to the measuring pendulum has indicated on the scale the tension in grams at which breakage of the clot had occurred.

with the next cuvette. The complete measurement takes about 30 seconds.

RESULTS

Only a few preliminary results will be quoted to illustrate the reproducibility of the method, and the range observed in a small series of normal and abnormal subjects.

NORMAL WHOLE BLOOD The cuvettes were filled directly from the syringe into which the blood was collected, and then incubated. It was usual to make the estimation one hour after clotting, but, in normal cases, longer incubation periods up to four hours had no apparent effect on the readings.

Reproducibility A series of 12 cuvettes was filled from the same sample of fresh blood and tested after one hour's incubation. The readings ranged from 5.3 to 6.3 g. with a mean of 5.8 g. and a standard deviation of 0.43 g.

Range in normal individuals Blood was obtained from 26 young adults, and tensile strength determinations were carried out on two or three cuvettes filled from each blood sample, the average for each individual being recorded. The figures ranged from 2.0 g. to 7.3 g. with a

mean of 4.48 and a standard deviation of 1.56 g. This series is too small to give more than a rough indication of the normal variation.

THROMBOTIC STATES Blood was obtained from 30 patients diagnosed as suffering from coronary, or deep vein thrombosis. Most were under treatment with dicoumarol drugs at the time of testing. The tensile strength determinations ranged from 2.9 to 13.0 g., with a mean of 7.24 and a standard deviation of 2.27 g.

There is thus a significant difference between the mean of this series and that of the normal range ($P = < 0.01$).

HAEMOPHILIA It was expected that haemophilic blood clots would be weaker than normal. Blood was obtained from six cases of severe haemophilia and though it was slow to coagulate, the clots once formed were, in four cases, abnormally strong, the figures obtained being as follows: 10.4, 22.0, 6.9, 3.5, 20.7, and 10.6 g., the mean for the group being 12.3 g.

DISCUSSION

The instrument described is simple to use and measures the tensile strength of blood clots with reasonable reproducibility. In its present form it is cumbersome, but, should further observations indicate the usefulness of the method, a smaller and more elegant version could be devised. Preliminary results suggest that wide differences in clot tensile strength exist in pathological states, though the significance of this cannot be judged until the factors responsible have been studied. It is possible, for example, that the increased tensile strength observed in cases of thrombosis and haemophilia is merely a reflection of an increased blood fibrinogen concentration. Many other factors, such as temperature during clotting, packed cell volume, number of platelets, age of clot, contact with glass and other surfaces, and the effect of therapeutic anticoagulants require investigation. As a further application the method might be used to measure the progress of fibrinolysis, and it could be adapted to measure the adhesion of clots to surfaces of different physical or chemical composition, giving information of possible relevance to the understanding of haemostasis, thrombosis, and embolism.

We are grateful to Mr. A. Lord and Mr. T. Strange for their practical help in constructing the tensiometer.

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An automatic staining machine for blood and marrow films on slides

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An automatic staining machine for blood films on cover-slips was designed by Davidson, Bareham, Kitchen, and Pegg (1958) and later developed commercially by Elliotts Liverpool Ltd. A modification of this machine was made by Elliotts Ltd. for this laboratory for the automatic staining of blood and marrow films on standard 3 in. $\times$ 1 in. slides, and it has been in routine use now for nine months with very satisfactory results. No mechanical or electrical faults have occurred.

GENERAL DESCRIPTION OF SLIDE STAINING MACHINE

Overall dimensions are approximately 20 in. $\times$ 20 in. $\times$ 13 in. high (Fig. 1). A maximum of seven different staining solutions can be used, each being contained in a standard glass trough (4 in. $\times$ 3½ in. $\times$ 2¼ in. high) and each of 200 ml. in volume. A maximum of 18 slides (3 in. $\times$ 1 in. $\times$ 1.0 to 1.2 mm. thick), in pairs back to back, can be stained in any one load. The slides are fitted into a simple stainless steel carrier which is hooked on to the undersurface of the plastic covering disc. The disc bears eight hooks so that the carrier can be hooked on to the disc in any of its eight positions.

FIG. 1. This photograph is of a machine with the aeration supplied to troughs 6 and 7 and not to troughs 5 and 6 as described in the text; also the safety device to prevent double staining as described in the text is not shown.

Received for publication 22 October 1960