Ultrasonic measurement of coagulation and fibrinolysis

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Since 1951 certain American workers have been using ultrasonic techniques in the study of blood coagulation (Yesner, Hurwitz, Rich, Roth, and Gordon, 1951; Roth and Rich, 1953; Levine, Luby, Rauch, and Yesner, 1954; Harrower, Hurwitz, and Yesner, 1958; Hurwitz, Yesner, and Cooke, 1952). The effect of intravenous heparin (Harrower *et al.*, 1958; Hurwitz *et al.*, 1952) and the influence of stress on the coagulation patterns of psychotic patients (Levine *et al.*, 1954) have been studied using this method, but apparently no other work has been done in this field and no comparison or correlation made with other methods. We have used an ultrasonic apparatus for the precise and continuous measurement of plasma and whole blood viscosity. The results obtained when the technique was applied to blood coagulation were compared with those from the thrombo-elastograph and it became apparent that it might usefully be applied to the study of fibrinolytic activity.

PRINCIPLE

Viscosity is that property of a liquid that resists flow. The original viscosity of whole blood changes rapidly when coagulation begins, and this change in viscosity can be followed continuously by means of the ultrasonic viscometer.² The apparatus consists of an ultrasonic generator and a probe, used in conjunction with a computer which gives a dial reading in terms of viscosity and density of the fluid being tested. The computer can also be connected to a recorder and in this way a continuous recording of the viscosity can be obtained (Figs. 1 and 2). The sensitive element is a 'blade' of stainless steel (Fig. 3), butt-welded to a length of magnetostrictive material. The blade is 4 in. long $\times$ 0.085 in. thick $\times$ 0.125 in. wide. It is silver-soldered near its centre to a diaphragm through which it passes, which is also the end of the probe shank. The section of the blade which is inside the shank is surrounded by a coil to which are fed electrical pulses from the generator. Each pulse magnetizes the blade and

decreases its length slightly (the magnetostrictive effect). This causes the surface of the blade to move slightly, longitudinally, shearing the fluid which is in contact with it. The maximum amplitude of the longitudinal motion is about 0.5 μ . The physical situation is exactly analogous to that of striking a bell or tuning fork with a hammer. The blade 'rings' at its natural resonant frequency of about 28 kilocycles per second. There is no motion at the diaphragm, which is a nodal point. The ringing vibration of the blade dies out more or less rapidly after each pulse, depending upon the viscosity of the fluid in which it is immersed. The exciting pulses are repeated approximately 50 times per second if the blade is in air. As the probe is immersed in more viscous fluids, the computer automatically responds to the increased damping by increasing the repetition rate of the pulses, so that the energy in the damped oscillations remains constant. This change in pulse-frequency, which is read from the meter, is proportional to the square root of viscosity times the density in Newtonian materials. It should be noted that while the pulse repetition rate changes with viscosity, the resonant frequency of the probe blade does not change. Furthermore, there is no lateral movement of the blade whatsoever.

MATERIALS AND METHOD

The probe was supported vertically in a rigid stand (Fig. 4), so that the blade was suspended in a small test-tube containing 4 ml. of blood. The tube was maintained at 37°C. by a thermostat and surrounded by an insulating box to reduce cooling. Parallel measurements with the thrombo-elastograph³ were made for comparison. In the experiments with fibrinolytic activators, 0.5 ml. of saline or saline containing the activator was immediately added to a 4.5 ml. blood sample in a disposable plastic syringe.

RESULTS

REPRODUCIBILITY The zero setting and calibration of the apparatus remained stable for at least 24 hours.

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²Ultraviscoson, manufactured by Bendix Corporation.

³Thrombo-elastograph according to Hartert (manufactured by the Hellige Co.).

FIG. 1

FIG. 2

FIG. 3

FIG. 1. General view of ultrasonic viscometer equipped for the study of coagulation and lysis of blood.

FIG. 2. Probe support specially designed for rapid and accurate manoeuvring. The probe is lifted.

FIG. 3. Ultrasonic probe: the sensitive part is 4.5 cm. long.

FIG. 4. Probe dipped into the reaction tube which is surrounded by a water jacket connected to a thermostat.

FIG. 4

and provided no mechanical stress was applied to the blade the equipment was completely reliable. Figure 5 shows duplicate recordings from two specimens.

COMPARISON WITH THE THROMBO-ELASTOGRAPH
Changes in viscosity are detectable with the ultrasonic technique earlier than with the thrombo-elastograph. Such changes are visible within two minutes of placing the blood in the tube. The maximum amplitude of the tracings by the two methods is reached at about the same time (Figs. 6, 7, and 8).

STUDIES WITH FIBRINOLYTIC ACTIVATORS Figure 6

shows the readings obtained after adding varying quantities of varidase to whole blood. Parallel thrombo-elastograms are also shown. Small quantities of varidase show no visible effect on the thrombo-elastogram and with larger quantities the trace is barely readable. The ultrasonic trace, however, shows readable changes at the lower concentration, while with the higher, a useful trace is still obtained.

Figures 7 and 8 show similar results using urokinase. As the concentration of activator is increased, the thrombo-elastogram shows dramatic changes, but when measured by the ultrasonic technique the changes are more progressive and more easily interpreted.

FIG. 5

FIG. 5. Reproducibility of ultraviscosonic measurements on whole blood.

FIG. 6

FIG. 6. Influence of varidase dissolved in physiological saline on coagulation of whole blood. Comparison between ultrasonic and thromboelastographic recordings on the same sample. All results obtained the same day on the same patient.

FIG. 7

FIGS. 7 and 8. Influence of urokinase dissolved in physiological saline on the coagulation of whole blood. Comparison between ultrasonic and thromboelastographic recordings on the same sample. All results obtained on the same day on the same patient.

FIG. 8

CONCLUSIONS

The kinetics of blood coagulation and fibrinolysis can be followed efficiently and in detail by this technique. It is particularly useful in the measurement of small variations and early changes which cannot be detected by other means. It is also well suited to the investigation of anticoagulants and fibrinolytic enzymes. No statistical conclusions can be derived from the results reported here, but they serve to demonstrate the usefulness of the method. It is not possible to make any statement about the viscosity of blood as such from these tests, as it is not known whether blood behaves as a Newtonian fluid under these conditions. While the readings

obtained may not represent actual viscosity, this does not detract from the practical usefulness of the results. In order to elucidate this point we intend comparing the clotting of blood with the clotting of such gels as agar and acrylamide.

SUMMARY

Ultrasonic techniques have been occasionally used to investigate blood coagulation, but the method has not come into general use. This method (Ultraviscoson-Bendix Corporation) has been applied to blood clotting and lysis in the presence of the fibrinolytic activators urokinase (Leo) and varidase (Lederle) and the results compared with thrombo-

elastographic recordings run in parallel on the same samples.

Urokinase, between 0 and 1,000 units dissolved in 0.5 ml. of physiological saline and added to 4.5 ml. of native blood, showed no effect on the thrombo-elastograph in low concentration. No reading was obtained in the higher range. The ultrasonic recording, however, showed changes sooner than the thrombo-elastogram and showed the onset of blood clotting even in the presence of large quantities of the lytic enzyme. Analogous results were observed with varidase between 0 and 320 units. As little as 14 units of urokinase per millilitre of blood can be demonstrated.

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DR. MACFARLANE asked how much Dr. Peeters' apparatus cost.

DR. PEETERS replied that the basic cost was about £900, and with a recorder about £1,200.

DR. STERN stated that he had studied psychotic patients with the Ultraviscoson and found that the results were affected by the adhesiveness of the clot. Platelet-rich and platelet-poor plasma gave differently shaped curves. He felt that, while the apparatus was extremely useful in homogeneous systems, it should be supplemented by other methods when studying coagulation phenomena in patients.

DR. PEETERS replied that he had overcome this difficulty by using smaller quantities of blood, better adhesion to the blade being obtained with small clots.

DR. LACK warned against investing hundreds of pounds in expensive apparatus, because he had information about a new method for measuring clot lysis, in which vibration is recorded from a crystal placed in the clot mixture. This apparatus should be extremely cheap.

DR. MACFARLANE then asked Dr. McNicol to give a brief account of units used in the measurement of fibrinolysis.

DR. McNICOL said that this was one of the fundamental problems facing workers in this field. Fibrinogen, for example, could be measured in a variety of ways: antigenically by the Hyland Fi test, as thrombin clottable fibrinogen, and as fibrin obtained by recalcifica-

tion, also as thrombin clottable fibrinogen after 24 hours' incubation in the presence of ϵ -amino caproic acid. Each method would give quite different answers, but probably the methods most relevant to this field were the use of thrombin or recalcification.

For the measurement of plasminogen, probably the best method available was the caseinolytic technique, but variations in the preparation of the casein would affect the results. At present there was no standard preparation of casein available and the standardization of this reagent would be most desirable. Similar problems were encountered in the assay of plasmin.

A satisfactory method for measuring anti-plasmin did not exist at present because there was no standard plasmin preparation free from contamination with activator. This means that any plasminogen present in the test solution will be activated, causing spurious results. There was at present no satisfactory method for measuring anti-activator, and this too would appear to be a field where methodological contributions remain to be made.

Finally the most difficult and important assay of all was that of activator. There are no really satisfactory techniques available for the assay of streptokinase, blood activator, or tissue activator, since all depend on the conversion of plasminogen to plasmin and therefore measure the plasmin so produced.

DR. MACFARLANE emphasized the difference between the behaviour of fibrinolysis *in vitro* and its possible behaviour *in vivo*, pointing out that laboratory experiments had no counterpart in the body. For example, nobody has passed blood over a surface for a great many years as happens in the circulation.

DR. EMERY felt that the object was perhaps to prevent clots forming rather than to lyse them after they had formed.

DR. STEWART, agreeing, stated that, in their experience, if activator is used quickly it can prevent fibrin formation, but that fibrin which has been deposited for more than a few days is extremely difficult to lyse.

DR. McNICOL stated that he had developed an artificial circulation made of glass, in which a clot could be subjected to a lytic solution pumped round the system. In those circumstances a clot, which would take days to lyse in a test tube, could lyse quite quickly.

DR. MACFARLANE reminded the meeting of the differences between a clot and a thrombus and asked what happened to a platelet thrombus when it was exposed to fibrinolytic agents?

DR. McNICOL stated that as a result of a few experiments it would appear that a platelet-rich thrombus, in fact consisting largely of platelet material, could be lysed *in vitro* with streptokinase.