Detection of Clot Retraction Through Changes of the Electrical Impedance of Blood during Coagulation

AMIRAM UR, M.D.

Clinical Research Center, Watford Road, Harrow, Middlesex HA1 3UJ, England

ABSTRACT

Ur, Amiram: Detection of clot retraction through changes of the electrical impedance of blood during coagulation. Amer. J. Clin. Path. 56: 713-718, 1971. The impedance method for the determination of the clotting time of whole blood has been found useful for the determination of clot retraction time. Although in previous attempts clotting and clot retraction affected the impedance in the same direction, the measuring cells used in the present work enable separation of the changes caused by the two processes. The introduction of disposable cells may enable easy and accurate determination of clot retraction time during routine impedance measurements of blood clotting time.

Within about 30 min. after formation of a clot, an increase in tension on the fibrin strands causes the clot to contract through an extrusion of serum and some of the contained cells. Superficially, the functional significance of clot retraction appears to be the increase in the tensile strength of the clot and the approximation of torn surfaces of blood vessels so as to reduce further hemorrhage and also reduce the cellular proliferation required to cover the defect. Because of the relatively small forces involved, however, the effectiveness of the process is doubtful, and it was suggested that clot retraction in mammals is a vestigial function related to the hemostatic mechanism in lower animals, which has been largely replaced by the more effective clotting process.

Clot retraction time and clot tensile strength are associated with normal platelet concentration and function. Budtz-Olsen showed also that over a range up to the normal concentration of platelets, clot retraction is almost a logarithmic function of the concentration of platelets. Patients with thrombocytopenia and thrombocytopenias usually have disturbed clot retraction, and the reduced clot retraction often associated with thrombocytosis is probably due to malfunction of the thrombocytes.
bocytes. The other major factor affecting clot retraction is fibrinogen, which is probably the cause for the disturbed clot retraction found in conditions such as cirrhosis.

Clot retraction has been measured in many different ways, dating as far back as 1819. Most methods involve measurement of the volume of serum extruded from a known volume of clotting blood within a specified interval of time. Budtz-Olsen developed an elegant technic for such measurement, in which blood is introduced into a suspending fluid of liquid paraffin and trichloroethylene: as serum is extruded it rises to the surface where its volume can be measured. Clot retraction has been measured quantitatively also, by thrombelastography. But in spite of the diagnostic information the test provides, busy physicians and laboratories avoid the tests, as they are laborious, time consuming and often have poor reproducibility.

In the search for an easy and accurate method for determining clotting time and clot retraction time, considerable efforts were made in monitoring the resistivity of clotting blood. It was presumed that the clotting would be associated with an increase in impedance, which would further increase with clot retraction because of the great difference between the resistivities of serum and blood corpuscles. Although the resistivity of blood is two to three times higher than that of plasma and serum, the resistivity of the packed cells themselves is as much as 30 to 40 times higher than that of plasma and serum. Thus, the progressive retraction of the clot would increase the specific impedance.

Although studies of impedance changes during coagulation started at the end of the last century, it was only in 1948 that Rosenthal and Tobias measured significant impedance changes in clotting blood. Their studies were followed up by Henstell, who demonstrated the possible diagnostic usefulness of impedance determination of clot retraction time in clinical conditions such as thrombocytopenic purpura, thrombocytopenia secondary to bone

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![Diagram](https://example.com/diagram.png)

**Fig. 1.** The measuring bridge circuit. $P_1 =$ reference (heparinized) cell; $P_2 =$ measuring cell; $R_1 = 15$ K Ohm resistor; $R_2 = 10$ K Ohm resistor; $R_3 = 10$ K Ohm 10 turn potentiometer; $C_1$ and $C_2 =$ variable capacitors of 250 picofarads; $A =$ 2 volts 10 KHz sinusoidal oscillator; $D =$ bridge output.

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marrow depression, Gaucher's disease,\textsuperscript{12} and in polycythemia vera before and after radiation therapy. Nevertheless, the technic was not adopted for routine laboratory use, because the impedance of the clotting blood was affected by many unrelated factors (e.g., temperature, sedimentation) which disturbed the measurements, and also because the variation in the initial impedance from one blood sample to another necessitated resetting the bridge for each test. Moreover, the increase in resistance caused by clot retraction follows the increase caused by the clotting process itself, making it difficult to distinguish accurately the two parts of the curve labeled by Henstell "primary" and "secondary" rise.\textsuperscript{10,11}

The difficulties associated with absolute impedance measurements are overcome in the present work by the new technic of comparative impedance measurements. This allows easy and accurate determination of clotting time, clot retraction time, and possibly, clot retraction intensity.

Materials and Method

The technic for comparative impedance measurements has been described in previous papers.\textsuperscript{27,28} The experiments were performed in an impedance coagulometer using standard glass cells (Stratton & Co., Hatfield, U.K.). The clotting blood sample, in a measuring cell, was placed in a bridge circuit (Fig. 1) in which the opposing arm consisted of another cell containing blood from the same sample but in which coagulation was prevented by a minute amount of heparin. By this means, all events in the blood sample, excluding coagulation, occur symmetrically on both sides of the bridge, and their effects therefore cancel out, leaving only the impedance changes caused by the coagulation process. There is also no need to adjust the bridge for each test, because variations in the initial impedance of different samples automatically cancel out.

The measuring cell and the identical reference cell consisted of glass capillaries with gold alloy electrodes plated coaxially onto each end (Fig. 2). The reference cell contained about 1 $\mu$g. of dry heparin to prevent coagulation. Because of its high molecular weight the heparin itself does not significantly affect the impedance. About 0.2 ml. of blood was needed to fill the two cells. The blood was obtained by venipuncture from healthy donors.
Results

The results of the tests are presented as automatically recorded curves (Fig. 3) showing the changes in the output voltage of the bridge with time. The bridge is initially set slightly off balance so that the output remains on one side of the balance point during the period of measurement. The clotting time corresponds to the minimum on the curve,\(^27, 28\) following which the bridge output increases to a maximum which correlates with the clot retraction time. This correlation was found by observing the column of blood in the measuring cell at a magnification of \(\times 20\). Until the time of the maximum, the separation of the blood corpuscles from the plasma was the same in both cells: it was slow, and the border between the two layers was hazy. With the appearance of the maximum, the separation of the two layers in the measuring cell became much faster, and the border became smooth and dense and assumed a concave contour. On the other hand, in the control cell, there was no significant change in the separation process. Following attainment of the maximum, the comparative impedance decreased, sometimes in a wavy curve, probably resulting from the uneven movements of the retracting clot as it became detached from the capillary walls.

Discussion

Although comparative impedance measurements enable accurate determination of clotting time, the specific design of the cells allows the isolation of changes caused by clotting from those caused by clot retraction. Thus, the clotting process is represented by an increase in impedance, and clot retraction is represented by a decrease.

![Fig. 3. The changes in the comparative impedance of blood during coagulation and clot retraction. The voltage scale is logarithmic.](https://example.com/fig3)

![Fig. 4. The electrical field in different cells used for monitoring impedance changes of blood during coagulation. (A, upper): a cell in which the electrodes are immersed in the clotting blood. The field lines pass through the clotting blood. (B, lower): a cell as described in Figure 2 above. The field lines concentrate at the periphery, where, after retraction, the clot is replaced by the relatively conductive serum.](https://example.com/fig4)
Although Rosenthal and Tobias,21 Henstell,10 and others found both processes to increase resistance, the results here do not contradict their basic observations, and the apparent difference is due to the specific structure of the measuring cells.

The cells used by Rosenthal and Tobias,21 Henstell,10-13 and others18 consisted of two electrodes immersed in clotting blood, so that the lines of the electrical field passed mainly through the clot (Fig. 4A). Hence, both clotting and clot retraction caused increases in the impedance of the cell. In contrast, in the cells used in the present work, the electrical field lines passed mainly at the outermost layer of the blood column (Fig. 4B) so that clotting, probably first activated near the glass surface, immediately increased the comparative impedance. With subsequent clot retraction and detachment from the glass wall, the electrical field lines pass mainly through a serum layer, of much lower resistivity, and so the comparative impedance of the entire cell decreases.

It was probably the inability to distinguish the part of the curve representing clotting from the part representing retraction that led Rosenthal and Tobias21 and also Molnar and colleagues18 to conclude that the increase in impedance was caused by clot retraction only. They deduced, therefore, that clot retraction began very early, even before it became visible, thus disputing Henstell's observation10 that increase in impedance occurred with the appearance of the first fibrin strands.

The surface properties of the cell strongly affect not only the blood clotting process, but also clot retraction.2, 7, 10, 11, 14, 16, 19, 20 Slight variations in surface characteristics can strongly affect clot retraction and prevent reproducible results. Accordingly, various methods for the cleaning of cells were devised, including, for example, flaming the cells to red heat.15, 16 In the present study, the cells were cleaned using a pipe cleaner and detergent solution (“Teepol”), then rinsed with distilled water and dried at 600°C. On some occasions, when cosmetic soap was used instead of detergent solution, the curves showed no minima, and the comparative impedance decreased through a plateau which appeared at about the time the minima were expected. In these cases the adherence of the clot to the glass surface was probably so feeble that retraction could start soon after clotting.

Future introduction of standard disposable cells may eliminate the variability in surface properties and enable accurate determination of clot retraction time and clot retraction intensity during routine measurements of clotting time.

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References


Addendum

Since the submission of the above paper, the method has been improved to allow finer determination of both clotting and clot retraction time and to demonstrate the reproducibility of the method and also to study the effect of intravenous administration of heparin on the impedance curve of blood during coagulation.

The accompanying illustration (Fig. 5) represents one of a series of tests performed on New Zealand White rabbits, using three impedance coagulometers and disposable measuring cells (Stratton Medical Ltd., Hatfield, Herts).

Blood was obtained from the lateral ear vein of the rabbit; the clotting time was determined simultaneously on the three impedance coagulometers. Following the appearance of the minima, M1, representing the clotting time, one of the coagulometers was stopped to allow an additional test while the other two progressed to show the maxima, CR, representing the clot retraction time. Meanwhile, 100 units of heparin per kg. of body weight were injected into the right ear vein and a second blood sample, collected 4 min. later (t2) from the left ear vein, was examined. The minimum (M2) of the curve representing the coagulation process following administration of heparin appeared 45 min. after collection of the blood, showing a clotting time eight times longer than that of the normal blood. However the general pattern of the curve remained unchanged, and it is impossible at this stage to detect any specific effect of the heparin with the exception of lengthening the curve. The clotting time of the heparinized blood was confirmed also in a control test similar to the one described in the above-mentioned work.

The scatter in the results of the normal clotting time was less than 30 sec. and it seems that this would further improve with the presently higher standardization of the measuring cells.

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