LI. A NEW CONDUCTIVITY METHOD FOR THE DETERMINATION OF THE CELL VOLUME OF BLOOD.

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MANY investigators [Bugarszky and Tangl, 1898; Stewart, 1899; Fraenckel, 1904; Gram, 1924; Fricke, 1924, 1, 2] have worked on the determination of the cell volume of blood by the conductivity method, improving that method but not succeeding in making it so accurate and simple that it could be commonly used. There is every reason to expect, however, that the conductivity method, so sound theoretically, when applied rightly will finally replace the haematocrit method, which is inaccurate and cannot be much improved.

The chief cause of the inexactitude of the conductivity method, in its present state, is the measurement of the conductivity of the whole blood. The blood as a suspension being often very unstable, its conductivity is also often very unstable; and yet it is necessary for accurate cell volume determinations to measure the conductivity of every sample of blood with the corpuscles each time in the same state of dispersion, if not in uniform dispersion, a condition which is very difficult to realise in practice. In a slowly settling blood, the corpuscles may be nearly equally dispersed while the conductivity is measured, but in a rapidly settling blood they are already agglutinated, vertically oriented and depositing; and these changes have a great influence on the conductivity. Stirring, besides being inconvenient, does not necessarily bring the corpuscles into a state of equal dispersion, neither does it always secure the same dispersion.

In the "two tubes" conductivity method, lately described [Slawinski, 1929; 1930], this inaccuracy is avoided by the measurement of settled blood, whose stability is much greater. A considerable simplification of the former method is also obtained by the use of two conductivity cells, instead of one, and the direct measurement of the ratio of two conductivities. This makes both the use of the thermostat and the necessity to separate the plasma for the measurement of its conductivity, as in the old method, unnecessary.

The conductivity cells are of the tube form, the only form practically indicated for the measurement of settled blood. A small conductivity tube, of about 0.5 cc. capacity, for human blood, is shown in Fig. 1.

The operations are conducted as follows.

Two conductivity tubes, closed at one of the ends with caps, are completely filled with blood from the same sample (by means of a dropping tube worked by a screw, for prompt and equal blood distribution between the two tubes), then closed at the other ends and placed, one tube horizontally and the other vertically,

in a holder, connected to a Wheatstone bridge. After a variable time (a few hours to 24), when the blood corpuscles have settled completely, we measure:

k , the ratio of the conductivity of the horizontal tube to the conductivity of the vertical tube, with the bridge;

h , the height of the blood deposit in the vertical tube, in relation to the height of the tube, with a telescope.

Fig. 1. Conductivity tube. t , graduated glass-tube; r , metallic rings, with threads, secured on the tube; c , metallic caps, with inner threads and platinum or platinized electrode bottoms, for the closing of the tube.

From these data the cell volume of blood is calculated by formula (4) given below.

The following formulae have been theoretically deduced for the method, and practically verified:

$$(1) \quad k_0 = \frac{\text{conductivity of plasma}}{\text{conductivity of dispersed blood}} = N + 1,$$

$$(2) \quad k_v = \frac{\text{conductivity of plasma}}{\text{conductivity of settled blood, in vertical tube}} = hN + 1,$$

$$(3) \quad k_h = \frac{\text{conductivity of plasma}}{\text{conductivity of settled blood, in horizontal tube}} = \frac{N+1}{(1-h)N+1},$$

$$(4) \quad k = \frac{\text{conductivity of settled blood, in horizontal tube}}{\text{conductivity of settled blood, in vertical tube}} = \frac{N^2}{N+1} h(1-h) + 1,$$

where

$$N = \frac{1.6438 + 0.1036 w_s}{1 - w_s} w_s,$$

$$w_s = \frac{w}{h},$$

w , cell volume of entire blood, per unit volume of the blood;

w_s , cell volume of the deposit of the blood corpuscles, per unit volume of the deposit;

h , height of the deposit in the vertical tube, in relation to the height of the tube.

The above function (1), as defined by the author, and similar functions k_0 of other investigators, are represented for comparison in Fig. 2.

The functions (2), (3) and (4), calculated for a cell volume $w = 0.4$, are represented in Fig. 3.

The curves for settled blood show that the conductivities of both tubes, just filled with blood and placed in the holder, are the same, but as the blood cells are sinking the resistance of the vertical tube k_v augments, that of the horizontal tube k_h diminishes, and the ratio $k = \frac{k_v}{k_h}$ increases. After 24 hours k equals

Fig. 2. Dispersed blood conductivity curves.

- Curve *A*, from Maxwell's [1892] theoretical formula for non-conductive suspensions.
 „ *B*, from Bugarszky and Tangl's empirical formula for the blood.
 „ *C*, from the author's theoretical formula for non-conductive suspensions.
 „ *D*, Gram's empirical curve for the blood.
 „ *E*, from Fricke's theoretical formula for the blood.
 „ *F*, Fraenckel's empirical curve for the blood.

Fig. 3. Settled blood conductivity curves.

$$k_v = \frac{\text{conductivity of plasma}}{\text{conductivity of blood in vertical tube}},$$

$$k_h = \frac{\text{conductivity of plasma}}{\text{conductivity of blood in horizontal tube}},$$

$$k = \frac{\text{conductivity of blood in horizontal tube}}{\text{conductivity of blood in vertical tube}}.$$

from 2.5 to 3.0, in the case of human blood; in the case of horse blood, it equals 6 or more.

For simplification's sake, formulae (1) and (3) can be discarded: formula (1) since it is but a particular case of formula (2), when $h = 1$, and formula (3) as having, for the present, no practical application. The remaining formulae (2) and (4), which represent methods (2) and (4), may be expressed as follows:

$$(2) \quad \frac{k_v - 1}{h} = N = \frac{1.6438 + 0.1036 \frac{w}{h}}{1 - \frac{w}{h}},$$

$$(4) \quad \frac{k - 1}{h(1 - h)} = n = \frac{N^2}{N + 1}.$$

Method (2) necessitates the use of a vertical tube filled with blood and a horizontal or vertical tube filled with plasma. The conductivities can be measured:

(a) When the cells are dispersed ($h = 1$), as in the old conductivity method. Since no thermostat is used, care should be taken that the temperature of both tubes, when the conductivity is measured, is the same. This method may be advantageously used in the case of a slowly settling blood.

(b) When the cells are well settled. This method will be seldom employed, having no practical advantages over method (4), given below, which is simpler.

Method (4) is the most advantageous in the case of promptly and moderately promptly settling blood. It necessitates, as said already, the use of two tubes filled with blood, one horizontal and one vertical, and the measurement of the conductivities when the cells are well settled. This method is very accurate with horse blood, and with human blood when the precipitation is accelerated. It is less exact with slowly settling blood, the concentration of the deposits (w_s) being probably in that case somewhat uneven, greater at the bottom of the deposits than at the top. In the case of very slowly settling blood, it is more advantageous to measure the conductivity of dispersed blood, using method (2).

For the calculation of the cell volume (w) by formula (2), we calculate N , as a function of k_v and h and then find w as a function of N and h ; we calculate similarly when formula (4) is used. Special tables make these calculations easy.

The conductivity tubes must be of accurate make, to allow an exact determination of the height of the blood deposit, the accuracy of this measurement being important. The line of separation of the red corpuscles is usually very sharp, when viewed by artificial light falling on the deposit from behind and from above. It is this line, and not the line of the white cells, often indistinct, that should be read. The determination of the ratio of the conductivities does not need to be so accurate when method (4) is used; but it should be most exact when dispersed blood is measured by method (2). One must remember that the temperature of both tubes must be the same at every measurement of their conductivity; hence this measurement should not be made just after standing for some time near the holder, or after any other heating of the tubes. The first condition of accuracy is, however, to have blood well protected against coagulation, to mix it well and to distribute it promptly between the two tubes. The latter is most important, of course, with rapidly settling blood.

The "two tubes" method can be applied to the accurate determination of the stability of cell suspensions [Slawinski and Pakowski, 1930; Labendzinski and Slawinski, 1931] and to the study of the permeability of the cell walls, by the measurement of the cell conductivity [Slawinski, 1930; Labendzinski and Slawinski, 1931]. As it allows a 0.1 % accuracy of the cell volume deter-

minations, it can be advantageously used to study the variations of the cell volume of blood in plasmata of different tonicities and for the calculation of the free water volume in the blood cells. Until now, most of these determinations have been made by the haematocrit method, which is inaccurate, and very different values have been obtained. Using the "two tubes" method, this determination becomes easy, and it has been found lately that the free water volume in horse blood cells is nearly 50 % of the cell volume¹.

A further application of the same method would be to study the concentration of ions in the cells and in the plasma, the distribution of urea between cells and plasma and the like.

SUMMARY.

This method measures the conductivity of settled blood, which permits a more accurate determination of cell volume than is possible by the measurement of unsettled blood by the old conductivity method. The simultaneous use of two conductivity cells enables the conductivity measurements to be made at air temperature and renders the use of the thermostat and the separation of the plasma for the measurement of its conductivity unnecessary. By these simplifications the conductivity method becomes almost as simple as the haematocrit method and has the advantage of being much more accurate than the latter.

The cells are of tubular shape and closed at their ends by metallic caps, which play the rôle of electrodes. They are entirely filled with portions of the same blood and placed, one horizontally and the other vertically, in a holder, connected to a bridge. The measurement is made after the blood corpuscles have settled completely (a few to 24 hours). The ratio of the conductivities of both tubes and the height of the deposit in the vertical tube are then measured. The cell volume is calculated as a function of these data.

By this method the cell volume of blood can be determined with an accuracy of 0.1 %, which renders possible an exact calculation of the free water volume in the erythrocytes and an accurate estimation of the distribution of substances and ions between the cells and the plasma.

¹ A report on these determinations will appear in the *Bulletin International de l'Académie Polonaise des sciences et des lettres. Classe de Médecine*.

REFERENCES.

- Bugarszky and Tangl (1898). *Arch. ges. Physiol.* **72**, 531.
 Fraenckel (1904). *Z. klin. Med.* **52**, 470.
 Fricke (1924, 1). *J. Gen. Physiol.* **6**, 375.
 — (1924, 2). *J. Gen. Physiol.* **6**, 741.
 Gram (1924). *J. Biol. Chem.* **59**, 33.
 Labendzinski and Slawinski (1931). *Compt. Rend. Soc. Biol.* **108**, 590.
 Maxwell (1892). *Electricity and magnetism*, **1**, 440. (Oxford, 3rd edition.)
 Slawinski (1929). *J. Chim. Phys.* **26**, 368.
 — (1930). *J. Chim. Phys.* **27**, 604.
 — and Pakowski (1930). *Compt. Rend. Soc. Biol.* **103**, 807.
 Stewart (1899). *J. Physiol.* **24**, 356.