BRIEF NOTE

Plastic Tube Technic of Centrifugal Separation of Leukocytes from Human Blood

By LENNART JUHLIN AND WALTER B. SHELLEY

THE ROLE of the leukocyte in disease is becoming increasingly evident. Indeed, a whole new area of immunohematology is rapidly being explored. This has, of necessity, called for new and simpler technics of isolating and handling the leukocytes. In this respect, Walford has given us the most detailed and comprehensive review of the methods for separating leukocytes from human blood. Many of the approaches rest on the fact that the leukocytes are lighter (specific gravity 1.070) than the erythrocytes (specific gravity 1.092) so that sedimentation or centrifugation in special chemical systems permits separation. However, in studies on the antigenicity as well as the cytology and physiology of the leukocytes, it would appear well to avoid the addition of chemicals to the blood. Accordingly, we have directed our attention to the centrifugal technics, which require only the addition of small and slight amounts of heparin to prevent coagulation. The use of a constricted centrifuge tube as first described by Bessis would seem an ideal answer. This has the disadvantage, however, of a relatively short segment of constriction which does not allow for the variable volume of erythrocytes in different specimens. As a result, the buffy stratum does not always appear in the constricted zone. To overcome this, Ottesen devised an ingenious "floating constriction" which by virtue of its specific gravity appeared at the proper level. An alternate method employed by Butler and Cushman involves the use of a set of four constricted tubes of variable size. It is clear that these technics pose special problems in sampling. It is therefore our wish to record here a simple plastic tube technic for the centrifugal separation and sampling of leukocytes from human blood.

DESCRIPTION OF METHOD

A 10 ml. sample of venous blood is drawn with a siliconized needle and syringe containing 10 units of heparin. This is immediately transferred to a special apparatus for centrifugation. Care is taken to minimize bubbling since this coats red cells with micro-bubbles and prevents sharp separation of the leukocytes and erythrocytes.

The centrifugation apparatus (fig. 1) consists of a 3-4 cc. glass flask and a glass funnel. The flask measures approximately 2.5 cm. at the base, stands 4
cm. high, and has a 4 mm. stem with a 2 mm. orifice. The funnel has a 3.5 cm. flare width with a maximum orifice of 2.0 cm. It measures 5.5 cm. high and tapers to a 4 mm. stem. These two siliconized glass pieces are connected by a 7 cm. piece of flexible, transparent vinyl plastic tubing (Tygon, S-22-1, 1/8" bore, 3/64" wall, Arthur H. Thomas, Philadelphia).

The assembled unit is filled with blood (using fine plastic tubing) to a volume indicated by the following formula:

\[
\text{Amount of blood} = \frac{\text{Flask volume } \times 100}{\text{Hematocrit per cent}}
\]

As shown in figure 2, the whole assembled unit is suspended in a 250 ml. centrifuge bottle. The funnel rim is protected by 0.5 cm. foam rubber pad and the unit is cushioned by partially filling the bottle with water.

After 15' centrifugation at approximately 2,000 rpm. the white cells will be found in a white column in the tubing. In first standardizing the relation of hematocrit and centrifuge time, it is necessary to vary the time and speed, but once the factors become apparent, it is possible to regularly place a well packed column of white cells in the plastic tube. If the cell mass is tinged pink, it is a sign of some erythrocyte contamination, commonly due to adsorbed air bubbles on the red cells.

The plastic tube is clamped with a hemostat well above the white cell column. Cutting just above the clamp, and also just above the flask with a

*Kindly made for us by Mr. James D. Graham, University of Pennsylvania.
PLASTIC TUBE TECHNIC OF SEPARATING LEUKOCYTES

Fig. 2.—Centrifugation unit showing white cell column in plastic tube as obtained using normal human blood. Slicing plastic tube permits precise fractionation.

Fresh siliconized Gillette Super Blue Blade (gentle sliding pressure, no sawing) gives a tube of cells which can be sliced into a number of strata for cell sampling or analysis. We have used the free hand razor technic, but a slicer mechanism sold for use with plastic tubing (Goldwater-Misco Centrifuge Tube Slicer*) or described for centrifuge tubes may be employed for exact work. For cytologic study, one may cut the clamped tubing at the lower end of the white cell column and make as many as 25 to 30 serial smears. Each smear made of successive “contact drops” thus reflects the ascending gradient of cell distribution.

The leukocytes layer in the definite patterning of their relative specific gravities with the lymphocytes most superficial and the granular leukocytes in the lower levels. A sample differential count of a smear from the uppermost and lowermost layers of normal human blood reveals how marked the separation may be:

<table>
<thead>
<tr>
<th>Layer</th>
<th>Polymorphonuclear leukocytes</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Top layer</td>
<td>2</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>Bottom layer</td>
<td>90</td>
<td>8</td>
<td>2</td>
</tr>
<tr>
<td>Circulating peripheral blood</td>
<td>39</td>
<td>39</td>
<td>2</td>
</tr>
</tbody>
</table>

*Microchemical Specialties Co., Berkeley, Calif.
The number of erythrocytes present varies, but may be as few as 1 per 10 leukocytes, indicating a ten-thousand-fold concentration of leukocytes.

Thus, it is possible with this method to rapidly secure sterile viable leukocytes suitable for experiments ranging from skin testing patients with lupus erythematosus to tissue culture work.

**SUMMARY**

A new simple plastic tube centrifugation method is described for the fractional harvesting of viable leukocytes from 10 ml of human blood.

**SUMMARIO IN INTERLINGUA**

Es describite un nove e simple methodo de centrifugation a tubos plastic pro le recolta fractional de viabile leucocytos ab 10 ml de sanguine human.

**REFERENCES**


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