METHODS

A Microhematocrit Method and Its Use with Citrated Blood

By Jean Captain Sabine, M.D. and Dorothy J. Nickolai, B.S.

Many microhematocrit methods have been described. In comparison to some, ours are only semi-micro, but are nevertheless suitable for finger or heelprick blood samples. They are cheap and durable, similar in design to standard Wintrobe tubes, and the objection that such tubes are difficult to fill and to clean has been overcome.

The anticoagulant of choice for small samples of human blood is dried heparin. The more rapid clotting of the blood of some small animals makes it difficult or impossible to get adequate mixing in time to prevent clotting. Citrate was preferred for the enzyme work which was done on the same samples, and for this reason the possibility of obtaining accurate hematocrit values on small samples of citrated blood was investigated.

Microhematocrit Tubes

These were made from heavy-walled capillary tubing of uniform bore with inner diameter about 0.8 mm., to admit freely a No. 20 spinal needle. The inside depth was 3 3/8 inches to allow a 3 1/2 inch needle to reach the bottom.

Blood Samples

For blood which can be successfully mixed with dry heparin, approximately 0.1 ml. is sufficient, since heparin does not distort the cells and the proportion of heparin to blood is not critical. If oxalate or citrate is to be used, somewhat larger samples are preferable, depending on the accuracy desired in the hematocrit determinations.

Filling and Cleaning

For filling, approximately 0.1 ml. of blood is drawn up into a small syringe through a No. 20, 3 1/2 inch spinal needle. The outside of the needle is wiped dry, and care is taken that no air is in the needle. (Air bubbles above the blood in the syringe do not cause trouble). The needle is inserted to the bottom of the tube, and a short, sharp push on the plunger fills the bottom part of the tube. Filling is completed by exerting gentle pressure on the plunger as the needle is withdrawn. A little practice is required for filling the tubes without bubbles but the knack is quickly acquired.

For cleaning, the tube is completely immersed in water, a spinal needle is
inserted to the bottom and connected to a vacuum pump. Water is pulled through until all the blood has been removed. Tubes can be dried in an oven, after as much water as possible has been removed by suction, or they can be filled with alcohol and then ether and dried by pulling air through the spinal needle.

**Fig. 1.**—The microhematocrit apparatus. The tube containing packed cells and plasma has been placed in the reader, the bottom being inserted into the hole in the lower strip of brass until the bottom of the cells is just tangent to the surface of the brass. The cell level (33.4) and the plasma level (76.8) are read from millimeter paper which has been mounted behind a thin layer of clear plastic. The hematocrit value, obtained by division, is 43.5 per cent.

**Centrifugation and Comparison with Standard Wintrobe Values**

The microtubes were placed in 15 ml. shields lined with heavy-walled rubber tubing of outer diameter to slip freely into the shield and inner diameter to fit the tube loosely. The tubes were stoppered with caps made from short pieces of rubber tubing cemented shut at one end. They were spun in a small clinical centrifuge at top speed for 45 minutes. This gave maximal packing and values within ±0.5 per cent of those obtained in standard Wintrobe tubes spun at 3000 r.p.m. for 30 minutes.
Reading the Hematocrits

The cell and plasma levels are read against millimeter paper in a special rack shown in figure 1, which was made from clear plastic and brass.

Standard Error

As shown in table 1, the largest value for standard error (σ) in ten sets of eight determinations was ±0.25 parts in 38, or ±0.66 per cent of the hematocrit value. The average of the ten values of σ was ±0.4 per cent. This is the standard error attributable to variations in the tubes themselves and in the reading, since the eight tubes were filled each time from the same sample of blood. It does not take into account errors caused by nonuniformity in handling the small samples of blood. The latter type of error has to be determined and controlled under individual experimental conditions.

Direct Comparison between Oxalated and Citrated Blood

Blood was mixed with oxalate or citrate in small beakers prepared by evaporating to dryness: (1) 0.1 ml. of Wintrobe's double oxalate solution1 per ml. of blood, or (2) 0.2 ml. of 2.4 per cent sodium citrate per ml. of blood. The 4.0 or 5.0 ml. level was marked on the side of the beaker. The rabbit blood was obtained by slitting the ear vein and collecting blood alternately into oxalate and citrate. The human blood was obtained by venipuncture and delivered from the syringe into the beakers.

The results are shown in table 1. Each value is the mean of eight determinations; “σ” is the standard deviation, ±√Σ(d²)/(n − 1). The “Ratio” was obtained by dividing the oxalate value by the citrate value.

<table>
<thead>
<tr>
<th>Species</th>
<th>Microhematocrit</th>
<th>Ratio</th>
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<tbody>
<tr>
<td></td>
<td>oxalate</td>
<td>citrate</td>
</tr>
<tr>
<td>Rabbit No. 1</td>
<td>40.48 ±0.14</td>
<td>36.83 ±0.16</td>
</tr>
<tr>
<td>Rabbit No. 2</td>
<td>46.01 ±0.28</td>
<td>41.73 ±0.10</td>
</tr>
<tr>
<td>Rabbit No. 3</td>
<td>43.72 ±0.24</td>
<td>39.81 ±0.21</td>
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<tr>
<td>Human No. 1</td>
<td>46.82 ±0.12</td>
<td>42.51 ±0.11</td>
</tr>
<tr>
<td>Human No. 2</td>
<td>38.04 ±0.25</td>
<td>34.33 ±0.07</td>
</tr>
</tbody>
</table>

SUMMARY

1. A method is described whereby accurate hematocrit values can be made from 0.1 ml. of blood using equipment which can be made easily and cheaply.
2. Under controlled conditions, a correction factor can be used to convert
hematocrit determinations on citrated blood to standard Wintrobe values. Under our experimental conditions the factor is 1.10.

REFERENCES

Methods: A Microhematocrit Method and Its Use with Citrated Blood

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