SCIENTIFIC APPARATUS AND LABORATORY METHODS

HEMATOCRIT DETERMINATION OF RELATIVE CELL VOLUME[1]

THE determination of the volume of erythrocytes and similar free cells relative to their suspending fluid may be carried out rapidly and with high precision. A number of types of hematocrit tubes have been described, all of which are adapted to low centrifugal forces. Adequate packing of red cells with

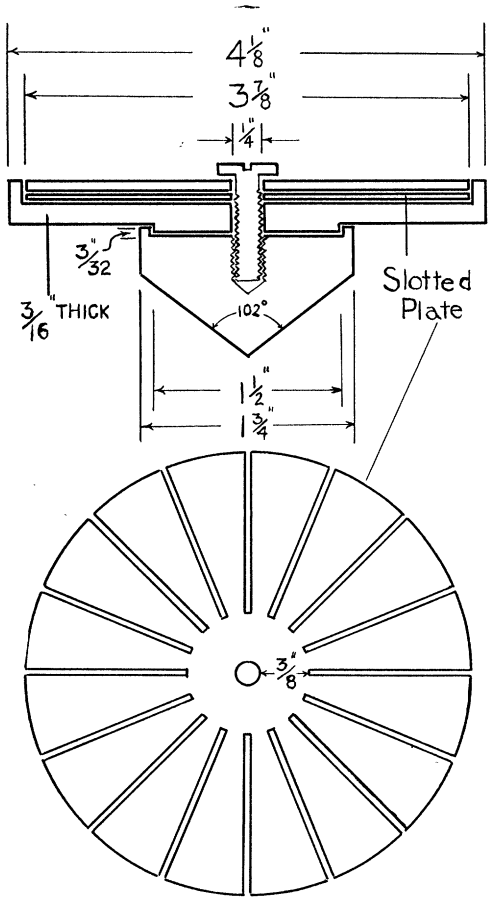

FIG. 1

complete expression of the supernatant fluid requires high forces. We have found thin-walled, capillary tubing (o.d. 0.5 mm, i.d. 0.35 mm) used in conjunction with an air turbine of the type used by Beams[2] a most satisfactory answer to this problem. The capillary tubing must have a uniform bore.

In detail the method involves allowing well-mixed blood to be drawn by capillarity into capillary tubes 3 cms in length to within 0.5 cms from one end. This latter end is quickly sealed in a small oxygen-gas flame, care being taken not to heat the blood. After brief practice at such sealing it is possible to seal the tube so that the internal bottom of the tube is approximately flat.

These filled, sealed tubes are put into the slots of the flat disc rotor shown in Fig. 1, and centrifuged for five minutes at a force of ca. 30,000 × G. This force and time of centrifuging was found to give practically complete packing of the red cells in freshly collected blood with a standard deviation of the red cell total volume of 0.34 per cent. This fact is based on experimental studies which have shown that at all forces between 20,000 and 190,000 × G, the degree of packing of red cells is approaching an asymptotic value which is 1.7 per cent. less than the packing obtained at the lower force. Between 40,000 × G and 190,000 × G the red cell volume drops only 0.7 per cent.

Studies on blood with varying degrees of hemolysis have shown that the relative volume of the intact cells can be determined by this method with a standard deviation of 0.84 per cent. This value does not include 3 deviations which were 3.7 σ, 5.9 σ and 6.2 σ; and is based on 106 determinations. The degree of hemolysis varied from 1 to 43 per cent.

Red cells which have been packed by this method can be resuspended and repacked three times without showing any change in the length of the cell column. This indicates that packing at high forces does not cause any rupture of the cells.

Readings on the length of the cell column and of the total column are made under a dissecting microscope against a standard steel rule engraved in 0.5 mm divisions. At the higher centrifugal forces short (1 cm) tubes are used. Their cell and total column length are measured under the microscope with a Filar Ocular Micrometer. Such readings can be reproduced with a standard deviation of 0.14 per cent. If longer times of centrifuging are required then these tubes must be sealed at both ends to check evaporation. Care must be taken to place the hematocrit tubes in the vertical position as soon after centrifuging as possible.

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[2] J. W. Beams, SCIENCE, 74: 44, 1931.