Cell Volume Determinations with Evans Blue (T1824). (18609)

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The determination of blood volume with Evans Blue(1) involves the determination of red cell volume. The value for the red cell fraction usually is found by use of the hematocrit, which may present certain disadvantages. Colorimetric methods have been published, and are in use, in which known amounts of Evans Blue, often as a dry residue in a tube, are added to known amounts of blood. After dispersion, and solution of the Evans Blue in the blood, the red cells are spun down, the absolute dye concentration in the plasma determined colorimetrically, and the relative volumes calculated. By this method, the hematocrit, with its various inaccuracies, is not used; but accurate, and often laborious, measurements of the Evans Blue to be added, must be made. Observation of spectral transmittance curves of hemoglobin and of Evans Blue solutions indicates that a large difference occurs in optical density of solutions of hemoglobin and of Evans Blue in the spectral region of 600-640 mμ (Fig. 1). It is on this basis that previous methods have been developed, in that small amounts of hemolyzed blood would have but little effect upon optical density measurements of Evans Blue. On the same basis it should be practicable to determine Evans Blue concentration in the presence of large amounts of hemolyzed blood. By making measurements of relative dye concentration upon a single sample of blood, that is, upon the plasma and upon the hemolyzed whole blood, one should be able to calculate relative volumes of plasma and whole blood from relative dye concentrations. The use of relative dye concentrations would make it unnecessary to know (to weigh or to determine) absolute amounts of dye in calculating cell volume. This method would then make it possible to determine cell volumes upon samples of blood taken for routine blood volume determinations using Evans Blue. Also, by addition of approximate quantities of Evans Blue to blood *in vitro,* the cell volume could be accurately measured. Assuming the validity of Beer's Law, the following relationships will pertain when working with a sample of blood containing Evans Blue, and a blank sample of the same blood containing no dye.

Plasma dye conc. = K (optical density of dyed plasma—optical density of blank plasma).

Hemolyzed whole blood dye conc. = K (optical density of dyed blood—optical density of blank whole blood), and (conc. of dye in whole blood)/(conc. of dye in plasma) = (vol. plasma)/(vol. of whole blood) = plasma fraction and cell fraction = 1—plasma fraction.

Procedure. Blood samples, oxalated or heparinized, are obtained in the usual manner for determination of blood volume using Evans Blue, that is, an initial sample is procured, a known quantity of Evans Blue solution, usually 2 ml of 1%, is injected by vein, and, usually 10 or 20 minutes later, a sample or series of samples is obtained. Of the initial blood sample, containing no Evans Blue, 0.3 ml are added to 2.7 ml of distilled water. The remainder is centrifuged and 0.3 ml of plasma are added to 2.7 ml of distilled water. The sample containing Evans Blue is treated in a like manner. After standing for 10 minutes to allow complete hemolysis to occur, the hemolyzed samples are centrifuged to remove suspended cell stroma. With the diluted initial plasma sample as a blank the optical density of the other three solutions is determined at 625 mμ. (In this case with a Beckman D.U. spectrophotometer). In the event that optical densities are found to be too high for accurate reading, the solutions may be diluted further or the wave length at which the readings are made may be increased to 640 mμ without invalidating the method of calculation. Plasma fraction =

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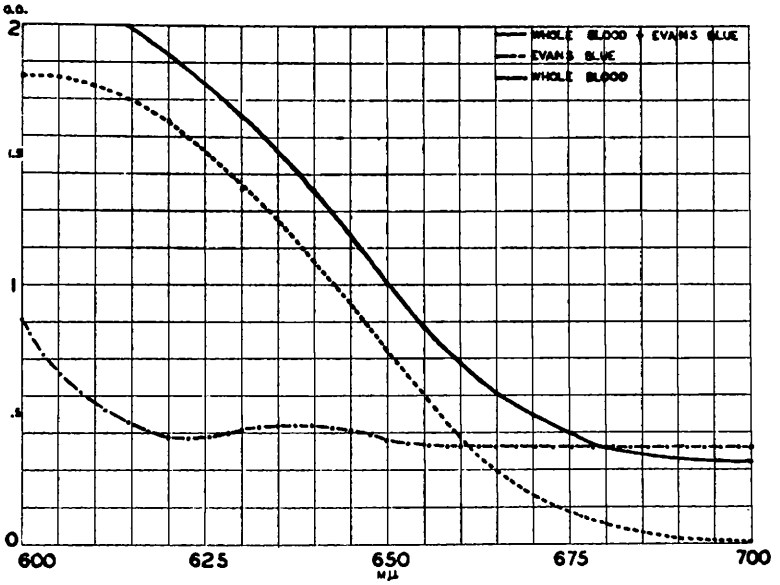

FIG. 1.

$(O.D._1-O.D._2)/(O.D._3)$ where $O.D._1$ = optical density of hemolyzed whole blood containing Evans Blue; $O.D._2$ = optical density of hemolyzed initial blood sample; $O.D._3$ = optical density of plasma containing Evans Blue (using dye free plasma as a blank).

Cell fraction = 1—plasma fraction. Routine blood samples, oxalated or heparinized, may be treated in the same way *in vitro*. The cell fraction is determined by dividing the blood sample into two parts and adding approximately 0.02 ml of 0.1% Evans Blue solution for each ml, to one part of the divided blood sample. A procedure, identical with that just described, is followed and cell fractions calculated.

TABLE I.

Cell vol. colorimetric method	Cell vol. corrected hematocrit	Difference
.375	.360	+.015
.392	.420	—.028
.421	.423	—.002
.288	.290	—.002
.415	.432	—.017
.395	.404	—.009
.412	.395	+.017
.427	.433	+.004
.334	.342	—.008
.338	.342	—.004
.427	.424	+.003

Results obtained by this colorimetric method were compared with those obtained by use of the hematocrit. The hematocrit was spun for 15 minutes at 3000 R.P.M. and a factor of .92 was used to correct for the volume of plasma trapped between the packed red cells. Experimental results are presented in Table I.

Summary. A colorimetric method is presented for the determination of cell fractions of blood. Accurate measurements of dye are unnecessary. The procedure may be applied *in vitro* or *in vivo*, in which case the routine samples drawn for determination of blood volume may also be used for the determination of the cell fraction.

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