A METHOD FOR THE DETERMINATION OF THE SPECIFIC GRAVITY OF RED BLOOD CELLS.

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In determining the specific gravity of red blood cells, the corpuscles alone should be studied. It is obvious that no direct information can be obtained from an investigation of whole blood, as the specific gravity of plasma is different from that of the cells. Two general methods for ascertaining the specific gravity of the corpuscles are available. One consists in weighing a known volume of red blood cells under constant conditions. Such a procedure, with cells completely freed from plasma, involves careful analytical work and is time-consuming. The second method is more simple and depends upon the use of two miscible liquids of different specific gravities, into which the sedimented corpuscles can be dropped. By varying the proportions of the liquids, a medium can be obtained in which the cells neither sink nor rise but remain suspended. The specific gravity of the red blood cells is the same as that of the mixture.

A suitable mixture must fulfill the following requirements. (*a*) The two fluids must be completely miscible in all proportions. (*b*) One must have a greater and one a lower specific gravity than red blood cells. (*c*) Neither individually, nor mixed, must these liquids dissolve any of the constituents of the red blood cells, or mix with them or enter into any chemical or physical reaction with the corpuscles. In other words, the liquids must be inert with respect to blood.

Roy (1) used mixtures of water and glycerol. Neither is fitted for specific gravity determinations because both extract the constituents of cells. Hammerschlag (2) suggested benzene and chloroform. Both of these dissolve lecithin and cholesterol from corpuscles. If whole blood is added to mixtures of chloroform and benzene, the plasma protects the red blood cells. The protection, however,

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lasts but a short time and the cells rapidly change in specific gravity (3). If red blood cells, freed from plasma, are used, they immediately become distorted in chloroform-benzene mixtures and soon hemolyze.

In searching for fluids which fulfill the requirements previously mentioned, benzyl benzoate was found to be admirably suited for the heavy liquid. It has a specific gravity of 1.115 at 20°C. and when mixed with sedimented corpuscles does not dissolve lecithin or cholesterol from them. If crystals of cholesterol are mixed with benzyl benzoate, they dissolve to some extent, but no acetic anhydride reaction is obtained when red blood cells are used. Chloroform, in which corpuscles have been suspended, gives a marked cholesterol reaction. A drop of sedimented cells maintains its shape in benzyl benzoate and shows no hemolysis in 24 hours. If the red blood cells are placed on a slide with a drop of benzyl benzoate and the cover-slip is sealed by vaseline, no microscopic change is evident in 24 hours.

For the lighter fluid, cottonseed oil was found to be most suitable. It is miscible with benzyl benzoate in all proportions. Neither lecithin nor cholesterol is soluble in it. No microscopic or macroscopic changes in the red blood cells are apparent when mixed with cottonseed oil. The oil used in this work had a specific gravity at 20°C. of 0.920.

If mixtures of benzyl benzoate and cottonseed oil are made up, varying from five parts each to ten parts of the heavy liquid and none of the oil, a series is obtained with specific gravities ranging from 1.017 to 1.115. A set of small Wassermann tubes containing 2 cc. of each mixture is prepared for each determination. A variation of 0.02 cc. of either fluid changes the specific gravity by 0.002.

The red cells are prepared by centrifuging defibrinated blood in small Wassermann tubes for 15 minutes at 3,600 revolutions per minute. The supernatant plasma is entirely removed by means of a nipple-capped pipette. The sediment appears homogeneously red under the microscope, no plasma being observed, and the supernatant fluid appears to be no more tinged by hemoglobin than the plasma resulting from standing. In other words, the cells appear to be intact after the vigorous centrifugalization. A drop from this thick sediment is used for the test.

Table I shows comparative results obtained with five specimens by the benzyl benzoate-oil method and pycnometer determinations. The latter were carried out in the usual manner. The pycnometer, calibrated with distilled water, was filled with whole blood and placed in the thermostat at 25°C. for 30 minutes. After drawing the blood to the mark, the pycnometer was dried and weighed. The same procedure was carried out with plasma. With the aid of hematocrit determinations the specific gravity of the red blood cells was then calculated as follows:

x = specific gravity of red blood cells.

$$\begin{pmatrix}\text{Per cent of red}\\ \text{blood cells in}\\ \text{whole blood}\end{pmatrix} x = \text{specific gravity of whole blood} - \begin{pmatrix}\text{Per cent of}\\ \text{plasma in}\\ \text{whole blood}\end{pmatrix}\begin{pmatrix}\text{Specific}\\ \text{gravity}\\ \text{of plasma}\end{pmatrix}$$

TABLE I.

Blood specimen.	Weight of whole blood.	Weight of plasma.	Specific gravity of whole blood.	Specific gravity of plasma.	Hematocrit determination. Per cent red blood cells.	Specific gravity of red blood cells calculated.	Specific gravity of red blood cells by benzyl benzoate-oil method.
	gm.	*gm.*					
1	1.3279	1.2838	1.0618	1.0269	52	1.0924	1.092
2	1.3126	1.2813	1.0498	1.0249	36	1.0941	1.093
3	1.3173	1.2806	1.0537	1.0244	41.4	1.095	1.094
4	1.3205	1.2819	1.0563	1.0254	45	1.0941	1.094
5	1.3188	1.2804	1.0549	1.0242	44.5	1.0931	1.092

The table shows that in every instance the agreement is within 0.1 per cent. This is very close when it is considered that the error in hematocrit determinations is often as high as 1.0 per cent.

Table II shows results obtained in a few routine tests. These results show the variations in a few cases. It is to be noted that the washed red blood cells have a higher specific gravity than corpuscles centrifuged from whole blood. This change is probably due to the obvious diffusions from the red blood cells when washed. Corpuscles in 0.6 per cent saline concentration swell, and in 1.3 per cent shrink. The passage of water is naturally accompanied by a change in specific gravity as demonstrated in the table.

When several properties of the blood are under investigation, it is desirable to use rapid and accurate methods. Opitz and Frei (4)

recently studied the relation between the specific gravity and sedimentation rate of the red blood cells, using the pycnometer method. For just such experiments is the benzyl benzoate-oil method especially fitted by its accuracy, rapidity, and simplicity. Several determinations can be carried out in one-fourth the time required to do one by the pycnometer.

TABLE II.

Blood specimen.	Specific gravity.				
	Red blood cells from defibrinated whole blood.	Red blood cells washed free from plasma with Ringer's solution.			Red blood cells washed free from plasma with Ringer's solution standing at 30°C. for varying intervals of time.
		In 0.9 per cent Ringer's solution.	In 0.5 per cent Ringer's solution.	In 1.3 per cent Ringer's solution.	
1	1.093	1.097			6 hrs., 1.097.
2	1.096				
3					½ hr., 1.090; 1 hr., 1.085; 2 hrs., 1.088; 3 hrs., 1.090; 4 hrs., 1.091; 5 hrs., 1.094; 6 hrs., 1.087.
4		1.096	1.069	1.113	
5		1.089	1.065	1.109	
6		1.092			
		1.090			
7	1.089	1.096	1.063	1.108	9 hrs., 1.091.
			1.061	1.107	
8	1.081	1.087	1.069	1.104	3 hrs., 1.089; 10½ hrs., 1.084.
			1.073	1.107	

CONCLUSIONS.

1. A simple method for determining the specific gravity of red blood cells is described.

2. It is more rapid than and as accurate as pycnometer determinations.

3. The marked chemical and physical interactions between corpuscles and suspension fluids in other methods are avoided.

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