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DETERMINATION OF DENSITY DISTRIBUTION OF RED CELL POPULATION

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ABSTRACT

A simple microcapillary method for the determination of the distribution of red cell population with different density (may be of different age) is described. It is highly reproducible, easy to perform and inexpensive. It may be used in the future for the study of cell aging process or cellular hydration.

INTRODUCTION

Although the specific gravity of whole blood or its cell fractions can be determined by direct weighing of a given volume the indirect methods are preferable. The principle of the indirect methods described (1-4) consisting of preparing a series of liquids of varying specific gravity on the surface of which a drop of blood or cells is allow to fall from a predetermined height. Various aqueous salt solutions and organic solvents including chloroform, benzol, bromobenzene, xylene, and copper sulfate. One of the method described by Reznikoff (5) is suitable for the determination of the specific gravity of red cells because of the liquids used, benzyl benzoate and cottonsed oil, dissolves any of the constituents of the red cells or having any chemical or physical reaction with the corpuscles. Balantine and Burford (6) have used mixtures of phthalate esters to separate protozoa, mammalian cells and bacteria practically free of the suspending medium. Danon and Marikovsky (7) utilized the above method in the determination of the density distribution of red cell population. We wish to report our finding of density distribution of red cells population from normal healthy adults.

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MATERIALS

Methyl phthalate (sp. gr. 1.189) and di-n-butyl phthalate (sp. gr. 1.0416) were used to prepare stock solution of specific gravity ranging from 1.062 to 1.138, with increments of 0.004 (Table I). The stock solution are stored in brown bottles with glass stoppers at room temperature.

TABLE I. Proportion of di-n-butyl phthalate (fluids I) and methyl phthalate (fluid II) used in preparing the battery of separating fluid.

Fluid in gms.		Specific	Fluid in gms.		Specific
I	II	gravity	I	II	gravity
34.0	73.2	1.138	60.1	41.0	1.098
36.0	70.0	1.134	63.1	38.0	1.094
38.0	63.3	1.130	66.6	35.0	1.090
40.0	60.0	1.126	69.1	32.0	1.086
43.0	57.8	1.122	72.1	29.0	1.082
46.0	55.8	1.118	85.6	27.0	1.078
48.0	52.0	1.114	86.5	24.0	1.074
51.0	50.0	1.110	92.1	21.0	1.070
54.0	46.2	1.106	88.3	14.3	1.066
57.0	43.5	1.102	90.6	11.5	1.062

DETERMINATION OF DENSITY DISTRIBUTION OF RED CELLS

Approximately 2 ml. of freshly drawn EDTA-treated blood were used. The tip of a capillary tube (for microhematocrit determination) is dipped into the separating fluid until approximately 5 mm. of fluid column is obtained. A series of capillary tubes containing columns of separating fluid with different specific gravity are prepared and placed horizontally. Each capillary is then filled with blood sample until the upper level of the oily liquid is about 10 - 15 mm. from the other end of the capillary tube. The dry ends of the capillary tubes are sealed by the modeling clay and centrifuged for 30 minutes at 12,000 xg in a microhematocrit centrifuge. After centrifugation, the percentage of red cells that have passed through the separating liquid is calculated (the columns of red cells below and above the separating

fluid equal to 100 percent).

RESULTS

Distribution pattern of red cell populations obtained from 10 normal healthy adults are shown in the following table.

TABLE II. Percent Distribution of red cell population (Healthy adults)

Specific gravity	A			B		
	Mean	S.D.	S.E.	Mean	S.D.	S.E.
1.126	0	0	0			
1.122	1.22	1.26	0.40	1.22	1.26	0.40
1.118	1.31*	0.88	0.29	0.47*	0.70	0.23
1.114	9.47	9.89	3.13	5.89	5.96	1.99
1.110	23.95	18.08	5.72	14.21	12.99	4.11
1.106	69.55	20.59	6.56	45.60	9.34	2.96
1.102	90.20	9.74	3.08	20.65	14.79	4.68
1.098	98.70	0.71	0.22	8.50	9.74	3.08
1.094	98.75	0.71	0.22	0.05	0.14	0.04
1.090	99.45	0.37	0.12	0.70	0.75	0.24
1.086	0	0	0	0.55	0.37	0.12
1.082	0	0	0	0	0	0

A = % of red cell population higher density than the given specific gravity.

B = % of red cell population with density between 2 given specific gravities as shown.

* : may be error due to technical difficultes in one sample.

COMMENTS

This method is highly reproducible with only minimal variations when left at temperature up to 5 hours (7) or 37°C for 60 minutes. Repeat examination one week latter also demonstrated only minor difference from the first examination results. Danon (7) has also found that red cells become havier (approximately 0.008 in 5 hours at room temperature). This artifact can be avoided by keeping the blood sample at 4°C and let it stand for a few mi-

nutes to reach room temperature before centrifugation. In our hand, the centrifugation time of 15 minutes is adequate. The results obtained from using the modeling clay to seal the hematocrit tube and those using heat to seal the tube are the same but the former method is more feasible.

Our unpublished results indicated that it is important to establish the normal distribution curve in different age groups. The effect of blood cell regeneration rate may play a role since it is known that the specific gravity of red cells increases with their age. This method may be useful not only to estimate the percentage of young red cell population but probably the effect or degree of intracellular hydration.

SUMMARY

A simple method for estimation of the percent distribution of red cell population with different density is described. An example of the results obtained from 10 healthy adults are illustrated.

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