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THE SIZE OF ERYTHROCYTE GHOSTS

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Summary

The volume of resealed erythrocyte ghosts formed during hypotonic hemolysis of normal human erythrocytes was measured by means of a continuous mean corpuscular volume analyzer. The final volume of resealed ghosts was 140.6 ± 15.2 fl. Strong correlations exist between the volume of ghosts and the initial mean corpuscular volume and mean corpuscular hemoglobin of the erythrocyte, and between the enlargement ratio and the mean corpuscular volume or mean corpuscular hemoglobin of the erythrocyte.

Exposure of red blood cells to a hypotonic medium results in the release of intracellular hemoglobin. Subsequently, the hemoglobin-depleted cells (ghosts) reseal [1—4]. Holes formed in the membrane by the hypotonic treatment are closed in the resealed ghosts as determined by studies with the electron microscope. However, little attention has been paid to the volume of resealed ghosts.

This paper reports on the volumes of cells before and after resealing as determined by means of mean cell volumes during changes in the tonicity of the medium [5].

2 μl of heparinized blood were mixed with 40 ml of Celluent (Toa Medical Electronics Co., Kobe), an iso-osmolar diluent, and the mixture was intro-

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Abbreviations: MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration.

duced into a continuous MCV analyzer (Toa Medical Electronics Co.). The mean cell volume was measured at 5-s intervals. After 15 s, 60 ml of distilled water were added at a flow rate of 0.5 ml/s [5].

The detection principle for cell volume is the same as that of the Coulter counter [7—9], except that the analyzer has a compensation circuit for the salt concentration and temperature. Therefore, the analyzer can express the correct cell volume under hypotonic conditions. This compensation function was

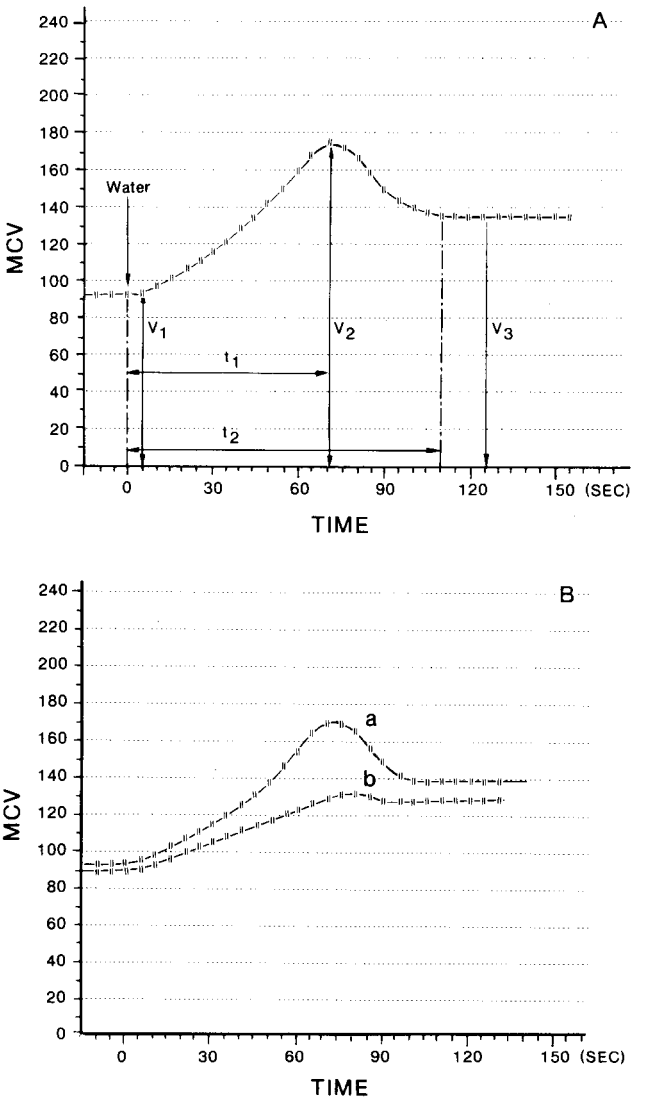

Fig. 1. (A) Fundamental pattern of hypotonic hemolysis curve. Sample blood of this figure was obtained from a healthy adult. Mean of cell volume was recorded at 5-s intervals at 20°C. Addition of water was started at 0 s on the figure. Flow rate of water was 0.5 ml/s. All curves were analyzed by time values, such as t_1, t_2, and by volume values, such as V_1, V_2, V_3, as shown in the figure. (B) Difference of collapse between curves a and b. This figure shows the examples of one curve with small collapse and the other curve with large collapse. MCV values of both samples were very close.

TABLE I

MEAN OF ANALYTICAL VALUE AND COEFFICIENT OF CORRELATION (r)

Results are expressed as mean ± S.D. (n = 226). RBC, red blood cells.

		Expansion volume (V_2) (fl)	Enlargement volume (V_3) (fl)	Reduced volume ($V_2 - V_3$) (fl)	Expansion ratio ($V_2 - V_1$)	Enlargement ratio ($V_3 - V_1$)
		164.5 ± 24.1	140.6 ± 15.2	24.5 ± 10.8	1.86 ± 0.15	1.60 ± 0.16
		Coefficient of correlation (r)				
MCV	(88.5 ± 13.4 fl)	0.81	0.73	0.81	*	−0.71
MCH	(29.9 ± 5.0 pg/cell)	0.73	0.65	0.80	*	−0.51
MCHC	(32.7 ± 2.0 g/dl RBC)	*	*	0.41	*	*

* $|r| \leqslant 0.4$.

checked under different conditions by using standard resin particles before each experiment.

Routine hemtological laboratory data were obtained by means of a Coulter electronic counter.

Volume changes during hypotonic hemolysis: During the addition of water to the cell suspension, the red cell volume increased gradually from its initial value (V_1, MCV) to the maximal volume, the so-called critical volume (V_2), and then decreased slightly ('collapsed') to a plateau level (V_3, enlargement volume) (Fig. 1). About 40 ml of water had been added when the maximal volume was attained and about 50 ml of water had been added when the plateau volume was reached. The plateau value remained constant even if the addition of water was continued. These swelling and shrinkage phenomena have been observed previously by measuring hematocrits [6,10]. The hemolysis curve measured directly, as shown in Fig. 1, was almost the same as that measured indirectly by the hematocrits. The time to reach the maximal volume was 76.9 ± 13.4 s, and to the plateau level 108 ± 16.5 s. The change from the maximal to the enlargement volume took about 30 s. Other analytical values are listed in Table I. Some cells showed substantial collapse and others showed little collapse (Fig. 1B, a and b). Seeman et al. [6] reported that there were some differences in the degree of collapse among the red cells, but did not attempt to interpret the observation.

The volume of resealed cells: The resealed cell volume (V_3) was 140.6 ± 15.2 fl and the enlargement ratio (V_3/V_1) was 1.60 ± 0.16. V_3 or V_2 and the initial MCV or MCH were closely correlated. The enlargement ratio was closely correlated with the initial MCV or MCH. On the other hand, the expansion ratio exhibited little correlation with MCV and MCH (Table I, Figs. 2 and 3).

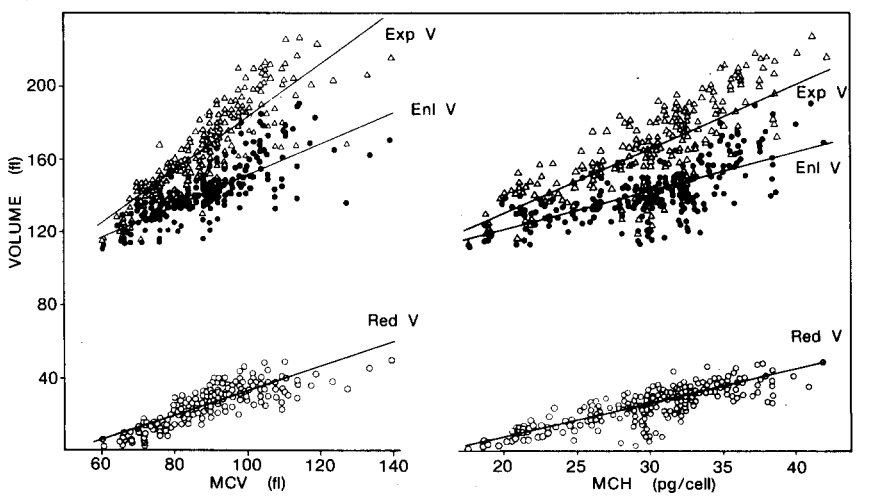

Fig. 2. Correlation between expansion volume, enlargement volume or reduced volume and MCV or MCH. (Left) △, MCV expansion volume (Exp V) ($y = 35.21 + 1.46x$, $r = 0.81$); ●, MCV enlargement volume (Enl V) ($y = 67.07 + 0.83x$, $r = 0.73$); ○, MCV reduced volume (Red V) ($y = -33.49 + 0.66x$, $r = 0.81$). (Right) △, MCH expansion volume ($y = 57.72 + 3.57x$, $r = 0.73$); ●, MCH enlargement volume ($y = 81.52 + 1.97x$, $r = 0.63$); ○, MCH reduced volume ($y = -27.31 + 1.74x$, $r = 0.80$). $n = 226$.

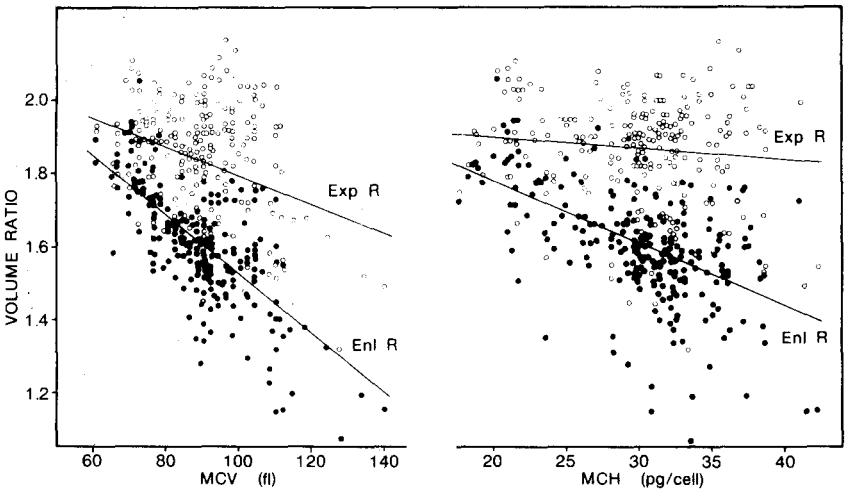

Fig. 3. Correlation between expansion ratio or enlargement ratio and MCV or MCH. (Left) ○, MCV expansion ratio (Exp R) ($y = 2.18 - 0.004x$, $r = -0.32$); ●, MCV enlargement ratio (Enl R) ($y = 2.33 - 0.008x$, $r = -0.71$). (Right) ○, MCH expansion ratio ($y = 1.96 - 0.003x$, $r = -0.10$); ●, MCH enlargement ratio ($y = 2.09 - 0.016x$, $r = -0.51$).

The volume of resealed ghosts was identical when the ratio of addition of water was changed from 0.66 to 0.066 ml/s. Therefore, the volumes are identical whether hemolysis is rapid or gradual.

However, the degree of collapse, which could be expressed as $V_2 - V_3$, showed a close correlation with MCV or MCH (Fig. 2). From the data, it can be concluded that the initial MCV or MCH determines the degree of collapse.

An interpretation of the data is that under hypotonic conditions microcytes develop into small resealed ghosts without showing collapse, and that hyperchromic macrocytes become large resealed cells that demonstrate considerable collapse. But, as most macrocytes were normo- or hypochromic, only a small amount of collapse could be observed.

The resistivity of resealed cells at V_3 is very stable, permitting accurate detection of cell volume at this time.

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