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Erythrocyte Volume Stability with Plasma Osmolarity Changes in Exercising Man[1] (37785)

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Shrinking and swelling of cells is based on the differential is osmotic potential of the intra- and extracellular medium whereby water can freely move through semipermeable membranes (1). The osmotic behavior of the erythrocyte has been studied in great detail (1, 2), and abnormalities in reaction to changes in osmolarity of its environment have been useful in clinical diagnosis (3). Most information concerning the osmotic properties of human red cells has been obtained from *in vitro* studies or specific pathological conditions (3–8). The procedure of correcting the relative red cell volume (hematocrit) *in vivo* for changes in plasma osmotic pressure with muscular exercise (9) assumes that the osmotic reactions of the human red cell *in vitro* are similar to those *in vivo*. It is unknown whether the plasma hyperosmolarity (10, 11) resulting from physical exertion affects the hematocrit readings and thus the calculation of proportional changes in plasma volume (12).

The present study was undertaken to evaluate the *in vivo* effects of changes in plasma osmolarity on the erythrocyte volume in exercising healthy human subjects.

Methods. Five male volunteers (22–42 yr old) participated as subjects in this study, performing maximal isotonic arm exercise of 8–10-min duration by cranking the pedals of an ergometer. Blood samples of 9 cc each were drawn from the antecubital vein 5 min prior to the exercise and within thirty and ninety seconds after the muscular activity. The proximity of the antecubital veins to the forearm muscles, which were predominantly involved in the physical work, provided for measurements of large changes in hematological parameters relevant to this study. All heparinized blood samples were immediately analyzed for acidity (pH) with an Astrup pH electrode maintained at 37.5°, and microhematocrit determinations were made in quadruplicate with an International Equipment Company capillary tube reader Model 2201 after centrifugation at 11,000 rpm for 10 min. In order to minimize exchange of metabolites between plasma and red cells after blood sampling, both the microhematocrit determinations and separation of plasma from the erythrocytes for chemical analysis were started within 2 min after collection of the blood samples. Red blood cell count (RBC) was performed in duplicate with a Model S Coulter counter, and the plasma osmolarity (P_{OSM}) was measured with an Advanced Instrument osmometer. The mean corpuscular erythrocyte volume (MCV) was calculated by dividing the hematocrit (Hct) by the RBC with the measured venous hematocrit corrected by 0.96 for trapped plasma: (Hct $\times$ 0.96)/RBC. The exercise was performed with minimal involvement of other muscle groups than those of the arms and the energy cost of the activity was evaluated by oxygen consumption (VO_2—STPD) measured with an open circuit technique (13). The expired gas was analyzed for O_2 with a Beckman E_2 analyzer, and for CO_2 with a Godart capnograph. The room temperature was 21° dry bulb.

Results. The obtained results presented in Table I indicate the large biochemical changes found in the blood of the forearm after

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TABLE I. Erythrocyte Volume During Variations in Plasma Osmolarity, Hemoconcentration, and Blood Acidity with Maximal Arm Exercise.

Subj.	Time	$\dot{V}_{O_2}$[a]	P_{OSM}	P_{OSM}	MCV	MCV	RBC	Hct	pH
	(min)	(ml/min/kg)	(mOsm/liter)	(Δ%)	(μ^3)	(Δ%)	($\times 10^6$/mm^3)	(%)	
E.B.	Pre-Ex		288.0		93.0		4.72	43.9	7.38
	Post-Ex$_1$[b]	21.3	300.4	+4.3	94.0	+1.0	5.08	47.8	7.21
	Post-Ex$_2$[c]		296.4	+2.9	93.4	+0.4	5.04	47.1	7.29
J.R.	Pre-Ex		300.0		84.2		5.20	43.8	7.37
	Post-Ex$_1$	22.9	329.5	+9.8	84.8	+0.7	5.66	48.0	6.99
	Post-Ex$_2$		314.1	+4.7	84.9	+0.8	5.53	47.0	7.17
W.B.	Pre-Ex		282.4		80.0		5.41	43.2	7.38
	Post-Ex$_1$	20.0	317.0	+12.2	80.7	+0.9	5.97	48.2	7.06
	Post-Ex$_2$		311.3	+10.2	80.6	+0.8	5.96	48.1	7.08
R.G.	Pre-Ex		296.0		86.3		4.77	41.2	7.37
	Post-Ex$_1$	25.4	318.4	+7.5	87.0	+0.8	5.19	45.2	7.12
	Post-Ex$_2$		316.5	+6.5	87.0	+0.8	5.18	45.1	7.15
J.P.	Pre-Ex		299.0		90.2		5.32	48.0	7.36
	Post-Ex$_1$	16.3	322.1	+7.7	90.7	+0.6	5.93	53.8	7.12
	Post-Ex$_2$		308.2	+3.0	90.5	+0.3	5.79	53.0	7.20
$\bar{X}$	Pre-Ex		293.0		86.7		5.08	44.0	7.37
±SE			3.3		2.2		.14	1.1	.00
$\bar{X}$	Post-Ex$_1$	21.1	317.5	+8.3	87.4	+0.7	5.57	48.6	7.10
±SE		1.5	4.8		2.3		.18	1.4	.03
$\bar{X}$	Post-Ex$_2$		309.2	+5.5	87.3	+0.7	5.50	48.0	7.18
±SE			3.5		2.2		.17	1.3	.03

[a] $\dot{V}_{O_2}$ = O_2 consumption during last minute of exercise.
[b] Post-Ex$_1$ = 30 sec postexercise blood sample.
[c] Post-Ex$_2$ = 90 sec postexercise blood sample.

the largely localized muscular effort. The measured changes in plasma osmolarity during this mode of exercise include the largest reported in the literature at the present time (11, 14, 15). With a mean increment of 8.3% in the plasma osmotic pressure, an average decrease of 4–6% in red cell size could have been anticipated (2). However, the mean erythrocyte volume (MCV) increased slightly (+.7%), which, considering the methodological errors in hematocrit determination (± .25%) and the RBC determination (± 2.0%), must be considered a nonsignificant change in the size of the red blood cell. Even an increment of nearly 35 mOsm/liter (+12.2%) in subject WB had no significant effect on the erythrocyte volume (Fig. 1). The increase in plasma osmolarity is apparently related to the exercise stress, considering the significant correlation ($r > 0.90$; $P < 0.05$) with the decrease in plasma pH.

Discussion. These findings are not in apparent agreement with the classical theory that red cells will shrink when placed in a hypertonic medium and behave as near-perfect osmometers (6). Since it has been demonstrated that cell volume changes resulting from osmotic differential pressures occur within milliseconds (6), the time component is probably not a factor in the present measurement of the MCV. It is possible that erythrocytes do not react to plasma osmolarity changes of 10–12%. However, Savitz *et al.* (6) measured *in vitro* significant changes in the red cell volume with osmotic changes of similar order as in our *in vivo* experiments. Evidently, experimental results obtained *in vitro* are in this case not directly comparable

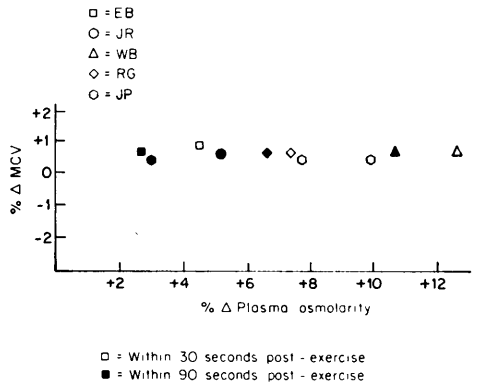

FIG. 1. Red cell volume with changes in plasma osmolarity during maximal arm exercise in men.

with *in vivo* conditions.

One explanation for the noticed discrepancy is that *in vivo* other events are occurring simultaneously with the shifts in osmotic pressures which can counteract the osmotic influences on the red cell. It has been noticed that, under conditions of chronic respiratory acidosis in man, the erythrocyte volume increases, presumably as the result of an increased hydrogen ion concentration gradient between the plasma and the erythrocytes combined with changes of metabolic events occurring at the site of the red cell membrane (8, 16). Consequently, hydrogen ions and also chloride ions, which are both highly membrane permeable, enter the erythrocyte and increase the osmotic potential of the intracellular environment (16). This elevation of the hydrogen ion concentration (see Table I) is probably the primary basis for the compensatory events in the red cell during exercise, counteracting the noticed increase in plasma osmolarity. The increase of the venous hydrogen ion concentration in the present experiments is undoubtedly related to the increase in CO_2 and lactic acid production with the strenuous activity of the forearm muscles (17).

To what extent other ions are involved in elevating the intra-erythrocyte osmotic pressure under the conditions of intensive muscular exercise is still open for speculation. It is known that two of the major metabolic end products of anaerobic activity, lactate and pyruvic acid, enter the red cells (18); however, this process is rather slow, requiring 10 min or more before an equilibrium between plasma lactate and red cell lactate concentration is established (19, 20). The fact that the human erythrocyte volume remains constant with muscular exercise suggests that lactic and pyruvic acid are not major factors in the regulation of the intra-erythrocyte osmolarity. Whatever the precise mechanism, the present data indicate that the events at the cellular level completely compensate for the significant augmentation in plasma osmolarity. At least three factors contribute to the significant augmentation in the osmolarity of the blood plasma.

1. A substantial rise of intravascular hydrostatic pressure, forcing out plasma water and thus causing hemoconcentration as evidenced by the 10% increase in hematocrit (12);

2. Elevation of tissue osmolarity facilitating water movement from the intra-vascular to the extravascular compartments (11); and

3. Transfer of osmotically active metabolic end products like lactic and pyruvic acids into the plasma.

Summary. Increasing the plasma osmolarity up to 12% with muscular exercise does not measurably change the volume of the erythrocyte in man. The results of this investigation emphasize that indiscriminate extrapolation of observations from *in vitro* studies to *in vivo* conditions may lead to erroneous conclusions. There is no evidence that the human red cell *in vivo* does not behave as an osmometer; however, its reactivity to osmotic changes does not necessarily result in volume changes because of other simultaneously occurring compensatory events. Consequently, the procedure of correcting the measured total relative red cell volume (Hct) for changes in plasma osmolarity should be used with discretion.

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