

# Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude

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*Simultaneous measurement of plasma volume and cell mass in polycythemia of high altitude.* J. Appl. Physiol. 28(6): 775-778. 1970.—In 13 native residents of Cerro de Pasco (4,330 m above sea level) simultaneous measurement of the red cell and plasma volumes was performed using the  $^{51}\text{Cr}$  and the Evans' blue dye techniques, respectively. A mean value of  $51.7 \pm 16.63$  ml/kg body wt for the red cell volume and a mean value of  $33.4 \pm 9.44$  ml/kg body wt for plasma volume were found. The fall in plasma volume in the majority of the cases had an inverse relationship with the hematocrit value, resembling the polycythemia seen in congenital heart disease.

hypoxia; hematocrit vs. plasma volume; venous hematocrit vs. body hematocrit

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TOTAL BLOOD VOLUME in polycythemia of high altitude has been measured by different authors. This total blood volume has been calculated from either the red cell mass or plasma volume, utilizing the venous hematocrit reading. Hurtado and co-workers (7, 8) and Merino (10) have used the plasma volume and venous hematocrit reading to calculate total blood volume. Lawrence et al. (9) and Reynafarje (cited by Sánchez-Medal et al., in ref. 12) have used both methods: plasma volume with the venous hematocrit reading and red blood cell mass to calculate the total blood volume.

Most investigators (1, 3-5) agree that when the red cell mass is estimated from the plasma volume, as determined by T-1824 dye and peripheral venous hematocrit, the red cell mass is greater than that obtained by direct measurement with  $^{32}\text{P}$  and  $^{51}\text{Cr}$ . Conversely, when the total blood volume and plasma volume are calculated by red cell tagging, the result will be erroneously low. This discrepancy appears to be due to the fact that the peripheral venous hematocrit is not representative of the hematocrit throughout the body.

Total blood volume is probably best determined by the addition of plasma and red cell volume directly and simultaneously estimated. Since the introduction of radioisotopes into clinical medicine, workers have been able to measure red cell mass and plasma volume separately.

The purpose of this paper is to report the values of plasma and red cell volumes estimated by direct and simultaneous method in a group of natives living at high altitude.

## MATERIALS AND METHODS

Seven healthy medical students, all males, whose ages ranged between 20 and 26 years, were chosen as the control group, each of them having resided many years in Lima (150 m above sea level). Thirteen healthy men, whose ages ranged between 20 and 54 years and who were permanent residents of Cerro de Pasco (4,330 m above sea level), represent the high-altitude group.

A simultaneous determination of red cell and plasma volumes was performed in each member of both groups.  $^{51}\text{Cr}$  (Rachromate-51, Abbott) as sodium chromate was used to estimate the red cell volume with the method described by Small and Verloop (13). Under sterile conditions 30 ml of blood was withdrawn from an antecubital vein into a sterile heparinized syringe and incubated with 30  $\mu\text{c}$  of  $^{51}\text{Cr}$  in a sterile glass bottle at room temperature for 1 hr with occasional stirring during that time. At the end of the incubation period 50 mg of vitamin C was added.

With a calibrated syringe, 20 ml of blood were reinjected into the subject intravenously, which left approximately 10 ml of blood in the bottle. From this, 1 ml was diluted 1:99 with distilled water immediately for calculation of radioactivity present in the injected blood. From another 2 ml of blood, a hematocrit determination was performed. The remainder of the blood was centrifuged and the radioactivity per milliliter of plasma determined. The amount of injected activity bound to the red cells was determined by subtracting the amount of activity in the plasma from total activity injected. The activity in the plasma was ascertained from the measured activity per milliliter of plasma and the hematocrit of the sample, which defined the number of milliliters of plasma injected.

One-half hour after the injection, a blood sample was withdrawn from the other arm. In this blood sample the hematocrit was determined and the radioactivity per milliliter of whole blood was counted; from the venous hematocrit the counts present per 1 ml of packed red cells were calculated. By dividing the number of counts present in the injected red cells by the count present per milliliter of patient's packed red cells, assayed in the sample withdrawn 0.5 hr later, the red cell volume could be calculated. The radioactivity of the blood specimen was measured in a well-type scintillation counter.

Using the same needle and a well-calibrated syringe, 10 ml of sterile 0.1% saline solution of Evans' blue dye were

carefully injected for the plasma volume estimation following the method described by Von Porat (15). Between 15 and 20 min after the injection of the Evans' blue dye solution, 10 or 15 ml of blood were collected from the opposite arm. This blood specimen was allowed to clot, then centrifuged, and serum was obtained without hemolysis. The dye concentration in the serum was measured with a previously calibrated Coleman Jr. spectrophotometer at 620 m $\mu$ . The calibration was performed using dilutions of different concentrations of Evans' blue dye with serum. A blank was performed by diluting the same serum with saline.

The individual's own serum before injection of Evans' blue dye was used as a blank. All of the dye solution used in this investigation was made from the same lot of Evans' blue dye. The plasma volume was calculated using the optical density at zero time extrapolated in millimetric paper from those obtained in the calibration curve.

The red cell and plasma volumes were determined while the subject was under fasting conditions and in the recum-

bent position for a least 0.5 hr before the performance of the test.

Venous hematocrit determinations were carried out in Wintrobe tubes centrifuged at 2,500  $\times g$  for 30 min. In the high-altitude group the venous hematocrit was centrifuged for 1 hr. This is a routine procedure carried out in our laboratory for many years. We believe that because of the increased viscosity of the blood at high altitude more accurate hematocrit readings are obtained when the blood is centrifuged for 1 hr. However, no great difference in red cell packing has been noticed with the different conditions of centrifugation (unpublished information).

An International centrifuge, size 2, model V, was used. The hematocrit readings, excluding the buffy coat, were corrected for trapped plasma with the method described by Chaplin and Mollison (2). Blood samples for the determination of both plasma and red cell volumes were taken using a tourniquet for the shortest possible time and aspiration of

TABLE 1. Findings in sea-level group (Lima, 150 m above sea level)

Subj	Age, yr	Wt, kg	TBV, ml	TRCV, ml	TPV, ml	TBV, ml/kg	RVC, ml/kg	PV, ml/kg	BH,* %	VH, %	BH/VH Ratio
1	24	62.0	4,464	1,689	2,775	72.0	27.2	44.7	37.8	38.6	0.979
2	24	71.0	5,215	1,682	3,533	73.5	23.7	49.7	32.3	43.0	0.751
3	26	78.0	5,763	2,473	3,290	73.9	31.7	42.1	42.9	44.4	0.964
4	25	61.0	4,378	1,921	2,457	71.8	31.5	40.2	43.9	43.4	1.011
5	20	54.5	4,303	1,628	2,675	78.9	29.9	49.0	37.8	42.5	0.889
6	26	77.5	5,942	2,150	3,792	76.7	28.0	48.9	36.2	44.8	0.808
7	24	64.3	4,802	1,672	3,130	74.7	26.0	48.6	34.8	39.3	0.885
Mean	24	66.9	4,981	1,888	3,093	74.5	28.3	46.0	37.9	42.3	0.898
$\pm$ SD			$\pm$ 672	$\pm$ 309	$\pm$ 484	$\pm$ 2.55	$\pm$ 2.87	$\pm$ 4.89	$\pm$ 4.47	$\pm$ 2.27	$\pm$ 0.090
Range	20- 26	54.5- 78.0	4,303- 5,942	1,628- 2,473	2,457- 3,792	71.8- 78.9	23.7- 31.5	40.2- 49.7	32.3- 43.9	38.6- 44.8	0.751- 1.011

TBV = total blood volume; TRCV = total red cell volume; TPV = total plasma volume; RCV = red cell volume; PV = plasma volume; BH = body hematocrit; VH = venous hematocrit. \*BH = (RCV, ml/(RCV + PV, ml))  $\times$  100.

TABLE 2. Findings in high-altitude group (Cerro de Pasco, 4,390 m above sea level)

Subj	Age, yr	Wt, kg	TBV, ml	TRCV, ml	TPV, ml	TBV, ml/kg	RVC, ml/kg	PV, ml/kg	BH,* %	VH,%	BH/VH Ratio
1	20	49.0	3,672	2,251	1,421	74.8	45.9	29.0	59.7	50.6	1.179
2	20	59.0	4,850	2,159	2,691	82.2	36.6	45.6	44.5	52.5	0.848
3	20	56.0	4,513	1,882	2,631	80.5	33.6	47.0	41.7	51.6	0.808
4	22	56.0	4,602	1,911	2,691	82.2	34.1	48.0	41.5	48.3	0.859
5	30	58.5	4,920	2,443	2,477	84.3	41.8	42.3	49.7	58.4	0.851
6	40	55.7	5,361	3,168	2,193	96.2	56.9	39.3	59.1	58.3	1.014
7	41	68.8	4,846	3,251	1,595	70.4	47.3	23.2	67.0	58.3	1.149
8	46	68.1	4,610	2,961	1,649	67.6	43.4	24.2	64.2	67.4	0.953
9	35	47.7	4,503	3,255	1,248	94.4	68.2	26.2	72.2	70.1	1.029
10	51	72.2	6,433	4,521	1,912	89.0	62.6	25.5	70.2	74.6	0.941
11	43	53.5	5,997	4,417	1,580	112.0	82.5	29.5	73.6	76.0	0.968
12	50	72.0	7,460	5,657	1,803	103.6	78.6	25.0	75.8	76.3	0.993
13	54	63.4	4,444	2,616	1,828	70.0	41.2	28.8	58.8	63.2	0.930
Mean	36	60.8	5,093	3,115	1,978	85.2	51.7	33.4	59.8	62.0	0.963
$\pm$ SD			$\pm$ 1,001	$\pm$ 1,138	$\pm$ 505	$\pm$ 13.39	$\pm$ 16.6	$\pm$ 9.4	$\pm$ 12.3	$\pm$ 9.9	$\pm$ 0.113
Range	20- 54	49.0- 72.2	3,672- 7,460	1,882- 5,657	1,248- 2,691	67.6- 112.6	33.6- 82.5	23.2- 48.0	41.5- 75.8	48.3- 76.3	0.808- 1.179

Abbreviations are the same as in Table 1.

the blood was started about 15 sec after removal of the tourniquet.

## RESULTS

Table 1 summarizes the various findings in the sea-level group. Mean total blood volume in this group was  $74.5 \pm 2.55$  ( $\pm$ SD) ml/kg body wt. Mean red cell volume was  $28.3 \pm 2.87$  ml/kg body wt and mean plasma volume was  $46 \pm 4.89$  ml/kg body wt. Mean BH/VH ratio was  $0.898 \pm 0.009$ .

Table 2 is a resume of findings in the high-altitude group. Mean total blood volume was  $85.2 \pm 13.39$  ml/kg body wt. Mean red cell volume was  $51.7 \pm 16.63$  ml/kg body wt and mean plasma volume was  $33.4 \pm 9.44$  ml/kg body wt. Mean BH/VH ratio was  $0.963 \pm 0.113$ .

Figure 1 is a graphical representation of the mean total and fractionated blood volume changes seen in the high-altitude group compared with the values found in the sea-level group. The white areas express the plasma volume and the black areas the red cell volume.

Table 3 shows the percentage change of the mean total blood volume, red cell mass, and plasma volume in the high-altitude group. As can be seen a +14% change in the total blood volume was detected and a +83% change in the red cell volume. On the other hand, a -27% change in plasma volume was recorded in this group.

## DISCUSSION

Our findings confirm what has been known since the first estimation of the blood volume was done in natives living in the high altitude, namely, the development of a polycythemia of the absolute type. In other words, there is a definite increase in the blood volume, due entirely to a rise in the red cell mass.

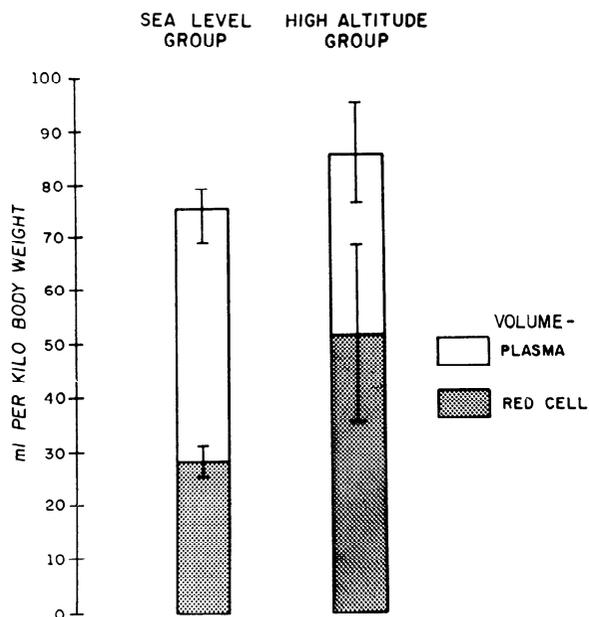


FIG. 1. Blood volume changes seen in high-altitude group compared with values found in sea-level group. White areas express the plasma volume and black areas the red cell volume. Bars represent standard deviation of the means.

TABLE 3. Percentage of variation of mean total and fractionated blood volume in high-altitude group

	Sea Level*	High Altitude*	Variation, %
Blood volume, ml/kg body wt	$74.5 \pm 2.55$	$85.2 \pm 13.39$	+14
Red cell volume, ml/kg body wt	$28.3 \pm 2.87$	$51.7 \pm 16.63$	+83
Plasma volume, ml/kg body wt	$46 \pm 4.89$	$33.4 \pm 9.44$	-27

\* Values are means  $\pm$  SD.

This polycythemia has been characterized as the result of increased erythropoietic activity as an adaptive mechanism to an environment of low  $PO_2$ , which by evidence accumulated during recent years exerts a stimulating effect on erythropoiesis indirectly through a plasma humoral factor, erythropoietin. It is well known, also, that this polycythemia does not affect the white blood cells or the platelets, and when the natives come down to a sea-level environment it disappears.

An analysis of the behavior of the two compartments of the blood volume seems interesting. Table 4 shows the values of plasma volume found by other authors in polycythemia seen in natives residing in the high altitude. As can be seen, our mean value is somewhat lower than the value given by Hurtado et al. (7, 8) and by Lawrence et al. (9). On the other hand the values given by Reynafarje (12) and Merino (10) are quite similar to our values. It is not clear why our values differ from those of Hurtado et al. (7-8). Several workers (12, 15) have found negligible differences between plasma volume estimates computed from a single sample at 10 min and those calculated by extrapolation from three to four samples. We feel that the lapse of 15-20 min to obtain the blood specimen is sufficient to give fairly accurate results of plasma volume estimates. The difference in values of plasma volume between our findings and those found by Lawrence et al. (9) may be explained on basis of the different methods used by this investigator. Reynafarje (12), however, using the same method ( $^{59}Fe$ ) found a plasma volume almost the same as that found by us. Hurtado et al. (7, 8) and Lawrence et al. (9) concluded that in polycythemia at high altitude the plasma volume is normal or slightly low. However, our values of plasma volume seem to indicate that this volume is somewhat low, and even more interesting, perhaps the main virtue of this paper, when the plasma volume is compared with the hematocrit a clear inverse correlation between these two parameters is noted (Fig. 2).

In this regard polycythemia at high altitude resembles polycythemia seen in patients with congenital heart disease. Work done by Verel (14) in patients with congenital heart disease shows a remarkably consistent fall in plasma volume as hematocrit values rise, with values of plasma volume of about half the normal value or less when the hematocrit is around 80%. Our work does not disclose the mechanism(s) by which the plasma shrinks; nevertheless, this decrease has to be within the physiological limits of viscosity that allow the blood to circulate through the blood vessels.

TABLE 4. Plasma volume in polycythemia seen in natives at high altitude

Author	No. of Cases	Method	Mean PV, ml/kg BW	Location, m Above Sea Level
Hurtado et al. (7)	20	Evans' blue	39.2	Morococha, 4,540 m
Merino (10)	7	Evans' blue	32.9	Morococha, 4,540 m
Hurtado et al. (8)	11	Evans' blue	36.2	Morococha, 4,540 m
Lawrence et al. (9)	11	<sup>59</sup> Fe	36.0	Morococha, 4,540 m
Reynafarje (12)	20	Evans' blue	35.0	Morococha, 4,540 m
Reynafarje (12)	10	<sup>59</sup> Fe	34.3	Morococha, 4,540 m
Present study	13	Evans' blue	33.4	Cerro de Pasco, 4,390 m

TABLE 5. Red blood cell volume (RBCV) in polycythemia seen in native residents at high altitude

Author	No. of Cases	Method	Mean RBCV, ml/kg BW	Location, m Above Sea Level
Hurtado et al. (7)	20	Indirect	61.1	Morococha, 4,540 m
Merino (10)	7	Indirect	67.2	Morococha, 4,540 m
Hurtado et al. (8)	11	Indirect	64.1	Morococha, 4,540 m
Lawrence et al. (9)	11	<sup>32</sup> P, <sup>59</sup> Fe	47.0	Morococha, 4,540 m
Reynafarje (12)	5	<sup>51</sup> Cr	52.0	Morococha, 4,540 m
Reynafarje (12)	10	<sup>59</sup> Fe	51.6	Morococha, 4,540 m
Present study	13	<sup>51</sup> Cr	51.7	Cerro de Pasco, 4,390 m

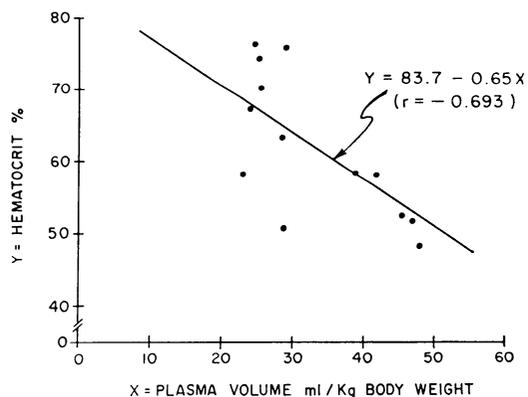


FIG. 2. Correlation between plasma volume and hematocrit values in polycythemia at high altitude.

Work done by Picón-Reátegui et al. (11) has shown practically no changes in body composition, as far as the total body water and extracellular fluid is concerned, of natives residing in Morococha when compared with data obtained

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in residents at sea level. It seems that at least the decrease in plasma volume is not due to shifts of fluids between the intracellular and extracellular space.

Table 5 shows the red cell volume found by other investigators in residents of Morococha. There is no question that an increase in this compartment takes place. Our mean red cell volume is less than that found by Hurtado et al. (7, 8) and Merino (10). We believe that this discrepancy is not due to the difference in altitude between Morococha and Cerro de Pasco, but to the difference in methods used for the determination of the red cell mass, as shown by Lawrence et al. (9) and by Reynafarje (12). These authors using direct techniques found, in residents of Morococha, a red cell mass quite similar to our values. Furthermore, it is known that higher values for red cell volume are obtained by using the indirect method as Hurtado and Merino did.

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