

Oxygen affinity of blood in altitude Sherpas

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SAMAJA, MICHELE, ARSENIO VEICSTEINAS, AND PAOLO CERRETELLI. *Oxygen affinity of blood in altitude Sherpas*. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 47(2): 337-341, 1979.—Oxygen equilibrium curves on blood within 6 h from sampling have been estimated from polarographic measurements of oxyhemoglobin concentration, in 13 male 14- to 50-yr old Sherpas residing at 3,850 m above sea level (Kumjung, Nepal). In samples with red blood cell counts = 4.7 ± 0.8 (SD) $\times 10^6/\text{mm}^3$, total hemoglobin concentration [Hb] = 17.0 ± 1.9 g/dl, and hematocrit = 53.3 ± 5.0 , the mean oxygen half-saturation of hemoglobin (P_{50}) (pH = 7.4 and P_{CO_2} = 40 Torr) was 27.3 ± 1.8 Torr. The P_{50} of altitude Sherpas was not significantly different from that of acclimatized lowlanders (28.2 ± 1.3 ; $n = 7$), sea-level Caucasian residents (26.5 ± 1.0 ; $n = 17$), and Sherpas at sea level (27.1 ; $n = 3$). The 2,3-diphosphoglyceric acid-to-hemoglobin concentration ratio ([2,3-DPG]/[Hb]) in altitude Sherpas was 1.22 ± 0.03 , the same as that of acclimatized Caucasians (1.22 ± 0.10). The Bohr effect measured for the blood of one altitude Sherpa by the ratio $\Delta \log P_{50}/\Delta \text{pH}$ was -0.32 and -0.45 at P_{CO_2} levels of 40 and 20 Torr, respectively. These values are not significantly different from those found in Caucasians at sea level where $\Delta \log P_{50}/\Delta \text{pH}$ was -0.35 and -0.42 , respectively. It is concluded that the P_{50} in native highlanders is not significantly different from that observed in sea-level dwellers. [2,3-DPG]/[Hb] at altitude, both in natives and in newcomers, is 20% higher than in sea-level residents.

oxygen half-saturation pressure of hemoglobin; 2,3-diphosphoglyceric acid; Sherpas; oxyhemoglobin dissociation curve; altitude natives; oxygen transport at altitude

SERPAS HAVE BEEN LIVING in the Himalayas of Tibet and Nepal for centuries. With the exception, perhaps, of some Andean communities living at altitude, the Sherpa is the only group adapted to hypoxia and well known for its outstanding performance in mountaineering and for carrying heavy loads at extreme altitude. Moreover, the physical working capacity and the endurance of the Sherpas have been found to exceed that of other Tibetan, Indo-Nepali, and Indian populations living for several generations in the same or in nearby regions as well as that of acclimatized Caucasians (10).

The consensus is, even though experimental evidence is lacking, that the Sherpa's average maximal aerobic power ($\dot{V}O_{2 \text{ max}}$) at a given altitude is higher than for Caucasians or other neighboring groups and/or that the relative drop of $\dot{V}O_{2 \text{ max}}$ caused by hypoxia is less. Several factors, both of physiological and biochemical nature, have been proposed to explain this behavior. Among these factors is an improved blood oxygen transport due to interactions between hemoglobin (Hb) and other mol-

ecules such as CO_2 and 2,3-diphosphoglyceric acid (2,3-DPG). However, a review of the literature on the subject of hemoglobin-oxygen (Hb- O_2) affinity of high-altitude natives from the Andes and the Himalayas leads to rather controversial results, as shown in Table 1. Apart from possible genetic differences among the investigated populations, these conflicting findings, particularly those concerning the value of oxygen half-saturation of hemoglobin (P_{50}), may be the consequence of ignoring or overlooking some variables known to affect the Hb- O_2 interactions. In addition, the way blood samples were collected, stored, transferred to the laboratory, and analyzed by different investigators may be the cause of some of the strikingly discordant results that from time to time have been published. Therefore, to gain some insight into the functional characteristics of the hemoglobin properties of high-altitude natives, a field laboratory was set up at Kumjung (3,850 m above sea level), a Sherpa settlement of the Khumbu Valley in Northeast Nepal. In addition, arrangements were made to collect blood from a group of Caucasians temporarily residing in the Western Alpine Range at an altitude of 3,500 m above sea level and from 17 healthy subjects at sea level (Milan, 122 m above sea level).

In all cases, all analyses except electrofocusing of hemoglobin were performed within 6 h from blood sampling, no matter whether blood was drawn in the field or in the laboratory.

MATERIALS AND METHODS

The experiments were conducted on a total of 40 male subjects: 13 Sherpas (14-50 yr old) permanently living and working at altitudes between 3,000 and 4,500 m above sea level (*group Sh_{nat}*); 7 Italians (22-29 yr old) working in an alpine resort, after a 3- to 5-day continuous sojourn at or following 2-8 mo of 8-h daily exposure to an altitude of 3,500 m (*group I_{acc}*); 17 nonacclimatized Italians (21-35 yr old) (*group I_{st}*); and 3 Sherpas (18-21 yr old) natives of Kumjung (3,850 m) after a sojourn of about 3 yr at 1,450 m above sea level followed by a further 3-mo stay at sea level (*group Sh_{st}*).

Blood samples were taken from the antecubital vein, heparinized, and stored at 0°C. All analyses were performed in duplicate and occasionally in triplicate.

Hemoglobin concentration, hematocrit (Hct), and red blood cell counts (RBC) were determined by standard laboratory procedures. [2,3-DPG] was determined by the end-point method of Rose and Liebowitz (18). P_{50} was estimated by a modified version of the method described

TABLE 1. P_{50} and $[2,3\text{-DPG}]/[\text{Hb}]$ at sea level and in acute and chronic hypoxic and altitude natives

Sea Level		Altitude, m	Acute Hypoxia (<4 days)		Chronic Hypoxia (>4 days)		Natives		Refs.
P_{50} , Torr	$[2,3\text{-DPG}]/[\text{Hb}]$, mol/mol		P_{50} , Torr	$[2,3\text{-DPG}]/[\text{Hb}]$, mol/mol	P_{50} , Torr	$[2,3\text{-DPG}]/[\text{Hb}]$, mol/mol	P_{50} , Torr	$[2,3\text{-DPG}]/[\text{Hb}]$, mol/mol	
24.4 ± 0.8		4,540	25.2 ± 0.8		26.1 ± 1.7		26.1 ± 1.7 (I)		Aste-Salazar & Hurtado (2)
26.0		4,000	27.6		26.6		26.6 (I)		Chiodi (5)
26.7		3,500	31.2		29.9				Mulhausen et al. (16)
26.6 ± 0.3	0.61	4,500	31.0 ± 0.4	0.94	27.3 ± 0.8	0.57	30.7 ± 0.6 (I)	0.80	Lenfant et al. (12)
28.3 ± 0.6 (I)	0.49								
	0.75	3,700				0.84			Eaton & Brewer (8)
26.8 ± 0.6		4,500			30.4 ± 0.9		30.6 ± 0.7 (I)		Lenfant et al. (13)
26.7 ± 0.1	0.94	4,300					29.8 (I)		Torrance et al. (22)
26.6 ± 0.3	0.91	4,500	30.6	1.15					Lenfant et al. (14)
26.2 ± 1.0		4,500	26.3 ± 1.2		27.5 ± 1.4				Weiskopf & Severinghaus (23)
									Cymerman et al. (6)
26.3	0.74	3,800	28.0	0.99					
Means									
26.3	0.76		28.5	1.03	28.2	0.90	28.8 (I)	0.80	
27.0 ± 1.0	0.88	3,900			30.0	1.26	22.6 ± 0.5 (S)	0.90	Morpurgo et al. (15)
36.7 (S)	1.36								

Values are means \pm SD. I, American Indians; S, Sherpas (Himalaya).

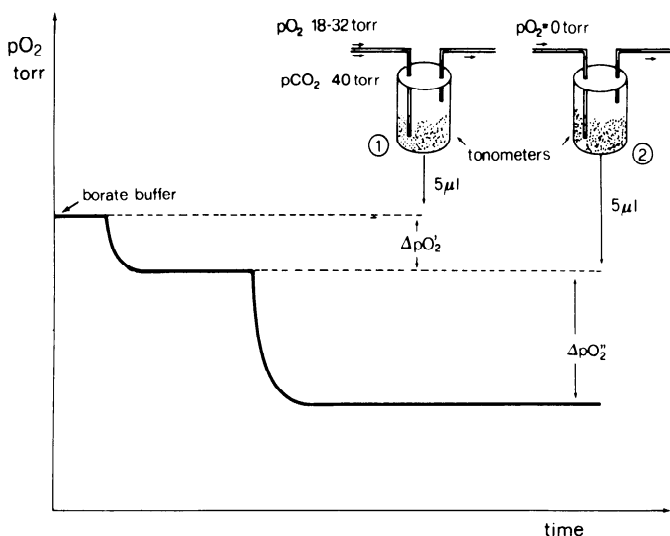


FIG. 1. Scheme of method used for estimation of oxygen saturation of blood at fixed P_{O_2} . Explanations in text.

by Rossi-Bernardi et al. (20). The following procedure, particularly suited for field measurements, was adopted. Two small amounts ($50 \mu\text{l}$ each) of the same well-stirred blood sample were equilibrated for 6 min in an AMT 1 Åstrup microtonometer (Radiometer, Copenhagen) at 37°C , one with a ternary gas mixture provided by a mixing pump (H. Wösthoff type 1 M 301/a-F, Bochum) (P_{O_2} range = 18–32 torr, $P_{CO_2} = 40$ torr, P_{N_2} to barometric pressure), the other with pure nitrogen (Fig. 1, top).

Hemoglobin-oxygen saturation was determined as

$$\% \text{HbO}_2 = \frac{[\text{total Hb}] - [\text{deoxy Hb}]}{[\text{total Hb}]} \times 100 \quad (1)$$

where $[\text{total Hb}]$ was the "active" Hb, i.e., all hemoglobin after subtraction of carboxyhemoglobin (HbCO) and

methemoglobin (metHb) and $[\text{deoxy Hb}]$ was the deoxygenated Hb.

The latter fraction was estimated by adding by a micropipette (SMI, Micropettor) $5 \mu\text{l}$ of the partially deoxygenated sample from tonometer 1 to 1 ml (37°C) of a 10-mM tetraborate buffer ($\text{pH} \approx 9$). The buffer, containing an hemolyzing agent (0.1% Sterox SE) was previously equilibrated with air in the anaerobic cuvette described by Rossi-Bernardi et al. (19). Adding a small amount of a partially deoxygenated blood sample to the tetraborate buffer resulted in a drop of the oxygen partial pressure (P_{O_2}) of the solution which was monitored by an O_2 microelectrode. This drop ($\Delta P_{O_2}'$) was proportional to $[\text{deoxy Hb}]$ and was due to the sudden oxygenation of deoxy Hb molecules following the shift to the left of the HbO_2 dissociation curve, prompted by the peculiar conditions of the sample, i.e., hemolysis, low $[\text{Hb}]$, and alkaline pH.

The addition of $5 \mu\text{l}$ of the N_2 -equilibrated blood sample from tonometer 2 to the same blood buffer solution caused a further drop of P_{O_2} ($\Delta P_{O_2}''$). $\Delta P_{O_2}''$ was proportional to the total $[\text{Hb}]$, inasmuch as the added blood was fully deoxygenated. The volumes and the $[\text{Hb}]$ of the two blood samples added were the same, and consequently any present HbCO and/or metHb would be the same in the two samples. Thus, Eq. 1 can be

$$\% \text{HbO}_2 = \frac{\Delta P_{O_2}'' - \Delta P_{O_2}'}{\Delta P_{O_2}''} \times 100 \quad (2)$$

from which the O_2 saturation at given P_{O_2} (e.g., in the range 18–32 Torr, as in Fig. 1) can be obtained. pH was determined by a microelectrode (BMS 3, Mk2, Radiometer) just after the second addition of blood. The pH ranges after tonometry (mean $P_{CO_2} \approx 40$ Torr) was 7.30 to 7.41 for group *Sh_{nat}*, 7.34 to 7.37 for *It_{acct}*, and 7.37 to 7.42 for *It_{st}*.

For %HbO₂ values from 30 to 70 approximately, P₅₀ was obtained by extrapolation from Hill's plot assuming an *n* coefficient of 2.6. Values of 2.6 for *n* were recently obtained by Winslow et al. (24) and also found for some of the subjects (both lowlanders and highlanders) appearing in the present study as a result of preliminary %HbO₂ determinations at two PO₂ levels, one below and the other above the expected P₅₀. It must be pointed out that the P₅₀ value obtained by the above procedure is not influenced by possible changes of [2,3-DPG] levels. In fact, it was observed (21) that an increase of blood 2,3-DPG up to 200% of its normal level affected the value of *n* less than 5%. Moreover, the experimental oxygen saturation in most cases (at least one of two or three analyses) was close to 50% minimizing the possible errors in the extrapolation.

To determine the Bohr effect, appropriate N₂-O₂-CO₂ mixtures were selected while the pH of the samples was changed by adding variable amounts of isotonic 0.1 M NaOH or 0.02 M HCl to the blood prior to tonometry. The Bohr effect was then calculated as the slope of a log P₅₀ vs. pH regression at constant PCO₂.

Electrofocusing of hemoglobin of some of the blood samples stored in ice for about 5 days were performed in the laboratory.

RESULTS

Table 2 summarizes all measurements. Compared to sea-level controls, RBC, [Hb], and Hct are higher in both high-altitude Sherpa natives and acclimatized Italians with no appreciable differences between the two groups. Moreover, high-altitude natives brought to sea level show a blood pattern similar to that of sea-level dwellers.

No significant differences in P₅₀ were found among the various groups. It must be emphasized that all the P₅₀ values of Table 2 refer to standard conditions of PCO₂ (40 Torr), pH (7.4), and temperature (37°C). These are not physiological values but indicate the "in vitro" oxygen carrying properties of blood that appears nearly unchanged under the various investigated conditions. While in high-altitude subjects (*Sh_{nat}* and *It_{acct}*) the P₅₀ was not significantly changed compared to sea level (0.1 < *P* < 0.2) the [2,3-DPG] was significantly (*P* < 0.001) higher.

In a single native Sherpa whose P₅₀ was close to the mean value for the group, the Bohr effect was measured in triplicate at constant PCO₂ values of 40 and 20 Torr, respectively. The log P₅₀/pH lines (not shown) appear to be shifted upward compared to sea-level controls. The

slope ($\Delta \log P_{50}/\Delta \text{pH}$) at PCO₂ = 40 Torr was found to be -0.35, i.e., not substantially different from that of a sea-level control (-0.32). The above values are similar to the standard value of -0.37 found by Wranne et al. (25) on normal individuals at sea level. At PCO₂ = 20 Torr, the $\Delta \log P_{50}/\Delta \text{pH}$ was -0.45 in the Sherpa and -0.42 in the lowlander.

The electrophoretic pattern of the Hb of the Sherpas indicates the presence of HbA and HbA₂ in the proportion normally found in Caucasians.

DISCUSSION

The blood pattern of native Sherpas at a given altitude does not differ significantly from that of acclimatized Caucasians except for their relatively low RBC for similar hematocrit. This observation confirms previous findings by Cerretelli (3) and Morpurgo et al. (15) on a different group of Khumbu Valley natives.

The results appearing in Table 2 indicate that 1) the mean standard P₅₀ of the Sherpas was the same as that of the controls; 2) the average [2,3-DPG]/[Hb] was markedly higher in Sherpas than in sea-level controls, although not different from that of acclimatized Caucasians; and 3) all blood variables of Sherpas at sea level were within the normal range for sea-level dwellers.

Oxygen affinity of Sherpa blood. Sherpas do not show the decrease of blood O₂ affinity described in Andean natives by Lenfant et al. (12, 13) as well as by Torrance et al. (22). The present data are in contrast with the results of the only other known investigation on Sherpas by Morpurgo et al. (15). These authors found in a group of seven individuals a remarkably higher blood O₂ affinity (P₅₀ = 22.6 Torr, see Table 1). Time from sampling seems to play a key role in these measurements. In fact, as recently shown by Aberman et al. (1) and Hellerstein and Buntharungroj (9), the P₅₀ of a blood sample decreases markedly after a few hours of storage even in an ice bath. The observed discrepancy between the P₅₀ values obtained in the present study and those observed by Morpurgo et al. (15) could be dependent on the different time between sampling and analysis elapsed in the two cases. This was less than 6 h for the present measurements as opposed to 5 days in the investigation by Morpurgo et al. (15).

2,3-DPG concentration, altitude hypocapnia, and the Bohr effect. The larger concentrations of 2,3-DPG, when expressed in a mole-to-mole ratio to Hb, found in the blood of both native Sherpas and acclimatized Caucasians, confirm previous observations made on Andean natives (22). Increased [2,3-DPG] prompted by the increase of [deoxy Hb] at altitude (7) decreases the O₂ affinity of oxyhemoglobin. This is an adaptive mechanism favoring O₂ release despite low blood O₂ content. It should be pointed out, however, that at altitude the physiological levels of P₅₀ are mainly the result of at least two counterbalancing effects: *a*) the increase of [2,3-DPG] and *b*) the decrease of arterial PCO₂ due to hypoxic hyperventilation leading to an increased O₂ affinity and therefore to a lower P₅₀. If the Bohr effect at the same PCO₂ is the same in Sherpas and in lowlanders as it appears from the determination carried out in one of the subjects of the present study, the interaction of the above

TABLE 2. Hematologic values and P₅₀ at standard conditions of four groups of subjects

	Sea Level		Altitude	
	<i>It_{nat}</i> (<i>n</i> = 17)	<i>Sh_{nat}</i> (<i>n</i> = 3)	<i>It_{acct}</i> (<i>n</i> = 7)	<i>Sh_{acct}</i> (<i>n</i> = 13)
[Hb], g/dl	15.5 ± 1.0	14.5	17.6 ± 1.2	17.0 ± 1.9
Hct, %	47.7 ± 3.0	46.0	50.0 ± 1.4	53.3 ± 5.0
RBC × 10 ⁶ /mm ³	4.71 ± 0.2	4.9	5.4 ± 0.3	4.7 ± 0.8
P ₅₀ , Torr (pH = 7.4; PCO ₂ = 40 Torr)	26.5 ± 1.0	27.1	28.2 ± 1.3	27.3 ± 1.8
[2,3-DPG]/[Hb], mol/mol	0.97 ± 0.09	1.1	1.22 ± 0.1	1.22 ± 0.03

Values are means ± SD. For explanations, see text.

mechanisms may lead to physiological P_{50} . In fact, in a Sherpa whose standard P_{50} value was found to be 28 Torr, the P_{50} was 26 Torr, when measured "in vivo" at pH = 7.4 and P_{CO_2} = 25 Torr. This in vivo value is within the normal range for nonacclimatized subjects.

Considering that the electrophoretic pattern of Hb also did not reveal the presence of abnormal hemoglobins, the above results rule out the hypothesis that the Sherpas could be characterized by a genetic or acquired facilitated O_2 transport mechanisms.

O₂ transport mechanisms, maximal aerobic power, and performance of the Sherpa. The outstanding performances of altitude Sherpas cannot be explained with an improved blood O_2 affinity. On the other hand, there is some recent experimental evidence (4) indicating that the Sherpas are not characterized, as it had been claimed, by a higher average specific (per unit body weight) $\dot{V}O_{2\max}$ than any acclimatized or even nonacclimatized Caucasian group.

Measurements of $\dot{V}O_{2\max}$ carried out at 5,350 m above sea level by one of us (4) on a group of 21 male natives of the Khumbu Valley of Nepal (3,200–3,900 m above sea level) yielded a mean value of 39.7 ± 7.1 (SD) $ml \cdot kg^{-1} \cdot min^{-1}$, a value not significantly different from 36.8 ± 4.5 found at the same altitude in a group of 32 well-acclimatized Italian amateur mountaineers. The $\dot{V}O_{2\max}$ of a group of 16 Sherpa natives of Kumjung (3,850 m), including most of the subjects whose blood pattern appears in Table 2 (*SH_{nat}*), was found to be 40.6 ± 7.7 $ml \cdot kg^{-1} \cdot min^{-1}$, not greatly different from the value of 37.4 ± 2.1 $ml \cdot kg^{-1} \cdot min^{-1}$ of 5 Italians and from the average value

of 39.4 ± 3.9 $ml \cdot kg^{-1} \cdot min^{-1}$ observed in a group of untrained unacclimatized Italians ($n = 26$) in a decompression chamber (4) at 3,750 m above sea level.

Also the mechanical efficiency of a familiar exercise such as stepping up and down from a 30-cm bench was found to be the same in 21 Sherpas and 15 Italians at 5,350 m above sea level (4). This fact rules out the possibility that the better performance of the Sherpas may depend on a more efficient mechanochemical coupling at the muscle level. There is the possibility, however, that in the Sherpas the muscle involved in locomotion may have a better O_2 supply at the expense of other muscles, e.g., those activating the ventilatory pump.

It is well known (11) that high-altitude natives ventilate less for a given work load. Even though the energy involved in the work of breathing appears to be in ordinary conditions only a small fraction of the overall energy consumption (17), it may become critical at the very high ventilation rates attained at extreme altitudes, thus improving the physical working capacity of the Sherpas compared to newcomers.

In conclusion, it appears that native Sherpas cannot be characterized by any special feature in terms of either blood O_2 affinity and/or overall $\dot{V}O_{2\max}$. This does not rule out the possibility that other factors of physiological (e.g., work of breathing) or perhaps psychological nature may actually improve their maximal performance, particularly at extreme altitude.

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