Red cell function at extreme altitude on Mount Everest

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WINSLOW, ROBERT M., MICHELE SAMAJA, AND JOHN B. WEST. *Red cell function at extreme altitude on Mount Everest.* J. Appl. Physiol.: Respirat. Environ. Exercise Physiol. 56(1): 109–116, 1984.—As part of the American Medical Research Expedition to Everest in 1981, we measured hemoglobin concentration, red cell 2,3-diphosphoglycerate (2,3-DPG), P_{O_2} at which hemoglobin is 50% saturated (P_{50}), and acid-base status in expedition members at various altitudes. All measurements were made in expedition laboratories and, with the exception of samples from the South Col of Mt. Everest (8,050 m), within 2 h of blood collection. In vivo conditions were estimated from direct measurements of arterial blood gases and pH or inferred from base excess and alveolar P_{CO_2}. As expected, increased 2,3-DPG was associated with slightly increased P_{50}, when expressed at pH 7.4. Because of respiratory alkalosis, however, the subjects' in vivo P_{50} at 6,300 m (27.6 Torr) was slightly less than at sea level (28.1 Torr). The estimated in vivo P_{50} was progressively lower at 8,050 m (24.9 Torr) and on the summit at 8,848 m (19.4 Torr in one subject). Our data suggest that, at extreme altitude, the blood O_2 equilibrium curve shifts progressively leftward because of respiratory alkalosis. This left shift protects arterial O_2 saturation at extreme altitude.

high altitude; acid-base; oxygen affinity; hemoglobin; 2,3-diphosphoglycerate; hypoxia

THE ERYTHROPOIETIC SYSTEM can participate in adaptation to high altitude in at least two ways: increased red cell number, and shifts in the position of the hemoglobin-O_2 equilibrium curve (OEC). The latter is controlled mainly by H^+ (the Bohr effect) and 2,3-diphosphoglycerate (2,3-DPG). CO_2 is an additional effector, but its physiologic importance under extreme conditions is less well defined.

A modest increase in the red cell mass is useful, physiologically, in increasing the O_2 carrying capacity of the blood (6). However, recent speculation has cast doubt about the quantitative benefit from this mechanism. Since increasing hematocrit leads to an exponential increase in blood viscosity, systemic and pulmonary pressures and resistance to flow could be undesirable consequences (8).

Whether a shift to the right or left of the OEC could be beneficial in hypoxia is another dilemma needing experimental clarification. Theoretical and animal studies (5, 22) suggest that a left shift would be beneficial at extreme altitude, but few data in humans are available. Although the OEC has been the subject of extensive experimental work at high altitude over the past half century, many of the results are of limited value because the effect of red cell 2,3-DPG concentration has been appreciated only recently (2, 4). This compound has a profound effect on hemoglobin-O_2 affinity, but since its concentration rapidly decreases in vitro, it must be measured within a few hours of blood collection. Obviously, immediate measurement is difficult in mountainous remote areas of the world.

The American Medical Research Expedition to Everest (AMREE, 1981) afforded a unique opportunity to study the red cell response and OEC at extreme altitude in 20 climbers and scientists. Our recent observations in the South American Andes indicated that sophisticated hematologic and acid-base measurements were possible, even under primitive circumstances (27). In addition, we had recently developed new instruments for field use that made measurements of 2,3-DPG, P_{50} (the P_{O_2} at which hemoglobin is 50% saturated), blood gases, and hematologic parameters possible, without the need to transport blood samples over long distances or time (15–18). This report summarizes the studies on Mt. Everest.

METHODS

Subjects. All AMREE members were males whose ages ranged from 26 to 52 yr old. The degree of physical training and climbing experience varied somewhat among the members of the group. Before the beginning of the expedition, all members were informed fully of the nature of the experiments and the risks of venipuncture; all consented to participate.

Laboratories and equipment. Studies were conducted in three principal locations: *1)* San Diego, California (May 1981), *2)* Base Camp (Sept 1981), and *3)* Camp II (middle and late Oct, 1981). The locations and elevations of these sites are shown diagramatically in Fig. 1. Care was taken to use the same techniques, instruments, and reagent stocks in each of the three locations. Instruments were packed and transported in special lightweight containers and carried at various times by ship, truck, plane, backpack, and yak. The laboratory huts and logistics of the expedition have been described by West (24). Blood gas and P_{50} measurements were not made at Base Camp
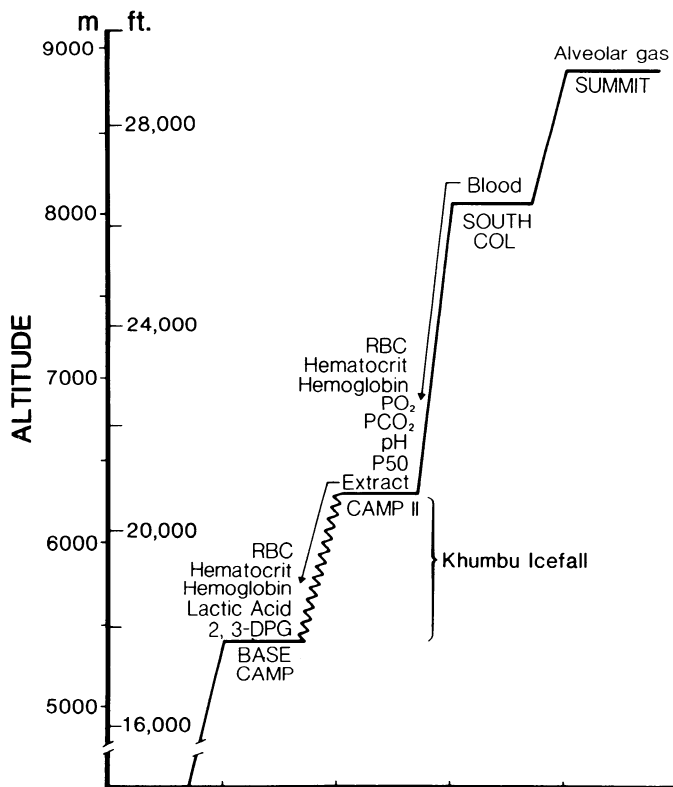
FIG. 1. Altitudes and locations of various activities. Laboratories were at San Diego, Base Camp, and Camp II. In addition to these locations, blood was obtained on the South Col; alveolar gas samples were collected on the South Col and summit. RBC, red blood cell; P_{O_2}, O_2 partial pressure; P_{CO_2}, CO_2 partial pressure; P_{50}, P_{O_2} at which hemoglobin is 50% saturated; 2,3-DPG, 2,3-disphosphoglycerate.

because the pH meter and a tonometer unit were damaged; they were repaired only in time for the Camp II studies.

Hematologic measurements. All venous blood samples were taken without stasis using plastic syringes with no. 20 needles. The anticoagulant we used was heparin that contained enough NaF to provide a final blood concentration of about 1 μmol/ml. This concentration of NaF effectively inhibits glycolysis in whole blood (unpublished observations). The samples were immediately placed on ice where they were kept until use.

Hematocrit measurements were made using a battery-powered Compur M1100 minicentrifuge. This instrument has a radius of centrifugation of 3.46 cm and a speed of 11,500 rpm. Each centrifugation cycle was 3 min, 20 s. The samples were subjected to repeated centrifugation cycles until the reading was constant.

Total hemoglobin concentration was determined in two ways. In San Diego and at Base Camp, 20 μl of whole blood were added to 5 ml of Drabkin's solution (Fisher Scientific) containing 0.1% Sterox. After 20 min, the absorbance at 540 nm was determined with a Perkin-Elmer model 35 spectrophotometer. A standard curve was prepared using various dilutions of International Standard hemoglobin solution no. 90500 from the International Committee for Standardization in Hematology, World Health Organization, kindly supplied by Dr. O. van Assendelft, Centers for Disease Control. Alternatively, hemoglobin concentration was measured using a

battery-powered Compur M1000 miniphotometer and the reagents supplied by the manufacturer. No difference in results could be detected by the two methods.

As a separate check of the hemoglobin measurements, 13 samples were drawn at Base Camp at the end of the expedition; hemoglobin concentration was measured and the samples were returned to Atlanta where the concentrations were measured using a Ljungberg hemometer.

Red blood cell counts were made with the Compur M1000 miniphotometer and the reagent kits supplied by the manufacturer. The above instrument measures the turbidity of a cell suspension; no effort was made to verify its accuracy. Both of the Compur instruments have been evaluated previously (7).

2,3-DPG. Measurements of red cell 2,3-DPG were made using kits from Boehringer-Mannheim (catalog no. 148334). These kits were packed in dry ice and hand-carried in insulated cartons to Katmandu and then by backpack to Base Camp. After the reagents were reconstituted, they were kept at ice temperature. The manufacturer's instruments for outdating the solutions were followed. Plastic disposable glassware was used throughout the procedures. Gilson micropipettors and tips were used to measure all reagents. Results are expressed as the [2,3-DPG]/[Hb] molar ratio.

At sea level, Base Camp, and Camp II, protein-free extracts were made within minutes of venipuncture, according to the manufacturer's instructions. The extracts were neutralized and stored on ice. Control experiments at Base Camp confirmed that the extracts were stable for up to 5 days. Extracts made at Camp II were carried through the Khumbu icefall to Base Camp in a Dewar flask containing an ice-water slurry, usually on the day after preparation; the delay was never longer than 2 days. The 2,3-DPG assays were carried out at Base Camp on the day of receipt.

Blood gases and acid-base status. Blood gas and acid-base measurements were made at sea level and Camp II using the same instruments, electrodes, and standards. The instrument was an Instrumentation Laboratories (Lexington, MA) model 213 blood gas apparatus fitted with Instrumentation Laboratories P_{O_2}, P_{CO_2}, and pH electrodes. Instrumentation Laboratories electrolytes and buffers were used throughout. Calibration mixtures of O_2, CO_2, and N_2 were obtained from Lif-O-Gen (Cambridge, MD), and their compositions were specified to a nominal ±0.02%.

Arterial blood was drawn at Camp II from radial arteries in three volunteers breathing either ambient air or a hypoxic mixture. The heparin-NaF anticoagulant described above was used, and the measurements were made immediately. Base excess was calculated using the algorithms of Thomas (21), modified to accept the O_2 saturation values.

Tonometry flasks. Tonometry flasks were filled within 3 days of use; they were filled at Base Camp for measurements at Camp II. These flasks, described in detail elsewhere (16), were sealed with rubber injection stoppers. Two no. 25 needles were used to introduce the gas, which was flowing at 250 ml/min for 10 min. The outflow was connected to a length of tubing submerged in H_2O to a level to insure that, at the end of the procedure, the

pressure in the flask exceeded ambient pressure by 20 cmH$_2$O. Later, the pressures were equalized by allowing some gas to escape when injecting the blood.

Hemoglobin saturation. We measured hemoglobin-O$_2$ saturation using an instrument constructed by Advanced Products (SRL, Milan, Italy) that is described separately (17). This consisted of a closed 1-ml cuvette containing 0.01% sodium borate, pH 9.1, 0.1% Sterox, and a small amount of antifoaming agent in equilibrium with air. An O$_2$ electrode with Instrumentation Laboratories electrolyte was in contact with the buffer. Lysis of the cells occurs when a sample of blood (10 μl) is introduced into the buffer. Because of dilution and the very high pH, hemoglobin becomes saturated with O$_2$, and the observed drop in Po$_2$ corresponds to the amount of deoxy hemoglobin present in the sample. After noting this drop, we added 5 μl K$_3$Fe(CN)$_6$ to oxidize the hemoglobin and release all bound O$_2$. The rise in Po$_2$ corresponds to total O$_2$ capacity, and the ratio initial drop/total rise is fractional saturation.

P$_{50}$ measurements. Blood P$_{50}$ measurements were made according to the general method described previously (15). Oxygenated fresh blood (250 μl) was injected into a tonometry flask that had been filled with gas mixtures of known O$_2$ and CO$_2$ composition to approximate the anticipated P$_{50}$ and arterial Pco$_2$. The samples were equilibrated with the gases in a tonometer for 20 min at 37°C.

The tonometer was modified from that previously described (17); the heater block was removed from its housing and placed into a styrofoam container for better insulation. The temperature of the blood was measured after each equilibration with a thermister probe (Yellow Springs Instrument, Yellow Springs, OH), kindly supplied by the manufacturer; it was specially calibrated by the factory before the expedition.

Since the Po$_2$ is chosen close to P$_{50}$, a logarithmic form of the Hill equation

$$\log (Y/1 - Y) = n \log Po_2 + C \qquad (1)$$

can be used to extrapolate to P$_{50}$. The slope, $n = 2.62$, corresponds to a 2,3-DPG/Hb ratio of 1.25 (16); slight variations in 2,3-DPG have little effect on this value (16). All of the calculations pertaining to saturation, Po$_2$, Pco$_2$, and P$_{50}$ are discussed in detail in previous publications (16, 17) and were performed on a Texas Instruments SR-62 pocket calculator.

RESULTS

Figure 1 shows, diagramatically, the locations and altitudes of our various activities. The transportation of red cell extracts down the Khumbu icefall between Camp II and Base Camp was a continuous problem because of limited personnel and weather uncertainty. Personal discomfort was considerable, particularly during the first 2 wk at Base Camp and the month at Camp II, but none of the operations or procedures was unreasonably difficult. Results of the programmed calculation of the saturation O$_2$ and P$_{50}$ results were extremely useful in monitoring results as data were taken.

Hematocrit and hemoglobin. Table 1 lists the values for hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration at the various locations. As expected, hematocrit and hemoglobin concentration increased over the period of exposure to altitude. Nevertheless, the hematocrit values were somewhat lower than those reported in previous studies at similar altitudes (3, 14). In anticipation of such problems, our methods were standardized before and after the expedition. The hematocrit minicentrifuge, with battery power, was evaluated at the Centers for Disease Control with conventional methods and was found to be accurate. In addition, samples from Base Camp were returned to the United States, and measurements were made using conventional methods; these studies confirmed the accuracy of the method (Fig. 2).

In general, persons whose hematocrits were in the lower part of the normal range at sea level remained in the lower range as altitude increased (Fig. 3). No correlation between absolute hematocrit, or relative change, and performance in climbing or success in acclimitization was noted within the group.

Mean corpuscular hemoglobin concentration. Mean corpuscular hemoglobin concentration (MCHC) increased in all members (Fig. 4). As with hematocrit, the increases were parallel and may reflect red cell dehydration, but other data related to hemoconcentration would be required to be certain.

Blood tonometry and acid-base status. Acid-base status was estimated or measured at several altitudes using the algorithms of Thomas (21); it was assumed that plasma protein changes did not affect the blood buffer base and that body temperature was 37°C in all cases. These algorithms allow consideration of the effect of hemoglobin saturation on the calculation of base excess. In all

TABLE 1. *Mean hematologic and acid-base measurements*

Altitude	HB, g/dl	Hct, %	MCHC, g/dl	2,3-DPG, mol/mol	Tonometry				
					pH	Pco$_2$, Torr	[HCO$_3$], meq/l	BE, meq/l	P$_{50}$, Torr
Sea level	14.5 ± 0.7 (18)	43.8 ± 2.3 (18)	33.2 ± 1.0 (18)	0.84 ± 0.12 (18)	7.399 ± 0.017 (17)	41.9 (17)	25.2 ± 1.0 (17)	−0.3 + 1.22 (17)	28.1 ± 1.1 (18)
5,400 m	17.8 ± 1.0 (20)	50.8 ± 2.6 (20)	35.1 ± 1.4 (20)	1.05 ± 0.09 (19)					
6,300 m	18.8 ± 1.5 (19)	53.4 ± 4.0 (18)	35.2 ± 1.1 (18)	1.04 ± 0.12 (17)	7.413 ± 0.034 (14)	22.6	14.1 ± 1.2 (14)	−8.7 ± 1.7 (14)	29.8 ± 2.2 (14)

Values are means ± SD for no. of measurements given in parentheses. Hb, hemoglobin; Hct, hematocrit; MCHC, mean corpuscular hemoglobin concentration; 2,3-DPG, 2,3-disphosphoglycerate; Pco$_2$, CO$_2$ partial partial; BE, base excess; P$_{50}$, Po$_2$ at which hemoglobin is 50% saturated.
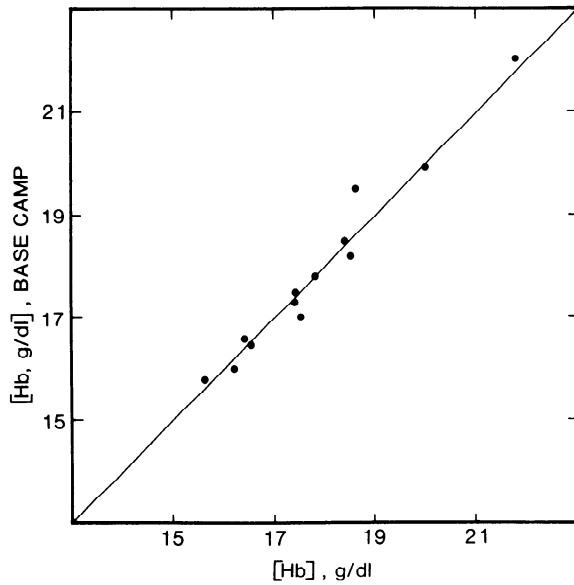
FIG. 2. Hemoglobin concentration. Fresh blood was drawn at Base Camp from 13 volunteers. Hemoglobin concentration was measured immediately using the Compur minispectrophotometer. These samples were then returned to Atlanta and measured again using the Ljungberg Hemomometer. Lines of identity are shown; $r = 0.982$.
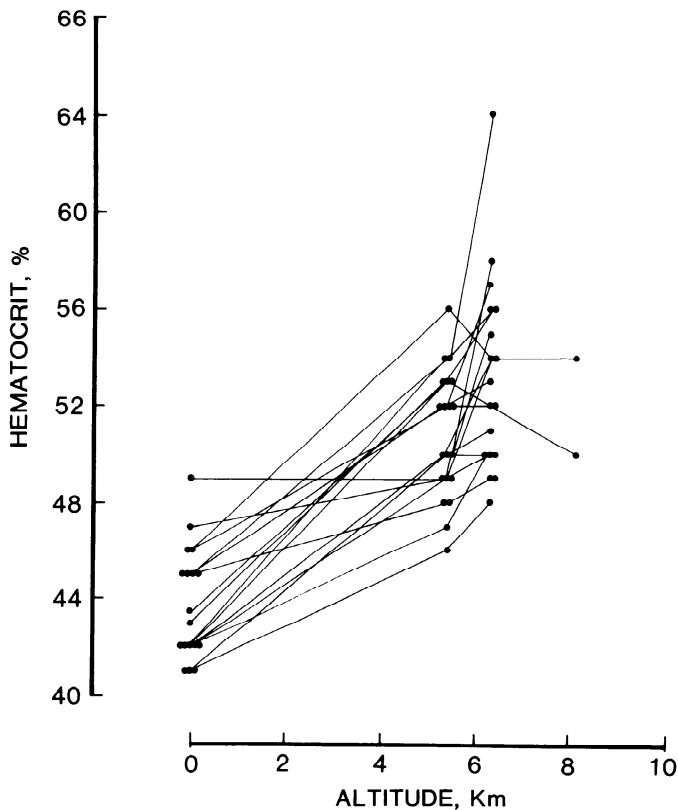

FIG. 3. Hematocrit values at various altitudes.

FIG. 4. Mean corpuscular hemoglobin concentration (MCHC) values at various altitudes.

cases, our calculations were conducted using the measured saturation after tonometry.

At sea level, the mean pH of equilibrated blood was 7.399 at P_{CO_2} 41.9 Torr and hematocrit 43.8; this corresponds to a mean base excess of -0.3 meq/l, a value which is within the error of the methods. Since the same techniques were used throughout our work, comparisons should be valid.
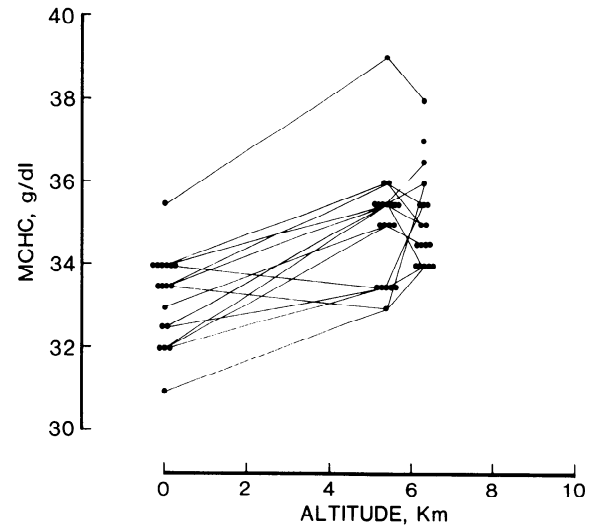
At Camp II, the mean base excess ($n = 14$) was -8.7 ± 1.7 meq/l measured by tonometry. In three subjects, arterial blood was sampled and base excess was calculated from direct measurement of pH, P_{CO_2}, and saturation, and after tonometry of the same samples (Table 2). The two methods gave similar results; base excess values were -6.7 meq/l for tonometry and -7.9 meq/l for the direct blood gas, pH, and saturation methods.

Blood drawn on the South Col from two subjects returning from the summit was subjected to the tonometry technique (Table 3). Their mean in vitro base excess was -7.2 meq/l, a value close to the mean for the Camp II group as a whole. It appears that compensation for respiratory alkalosis for one of these subjects (CP) was less effective than it had been earlier. On Oct. 8, his base excess of Camp II was -9.8 meq/l, whereas on the day after reaching the summit (Oct 25) his base excess was -5.9 meq/l (Table 3).

In vivo acid-base status on summit. Since direct sampling of arterial blood on the South Col or summit was not possible, in vivo pH must be inferred from the tonometry data. This was accomplished as follows. Venous blood was sampled from two climbers on the South Col (Table 3) about 12 h after returning from the summit. The samples were taken to Camp II and equilibrated to a final P_{CO_2} of 22.6 Torr. The pH was measured, and BE was calculated using the Thomas algorithms (21). To estimate the summit pH, in one of the climbers (Table 4) we then used the alveolar P_{CO_2} measured on the summit (7.5 Torr, Ref. 25) and assumed arterial P_{CO_2} = alveolar P_{CO_2}. To construct an in vivo buffer line on the Sigaard-Anderson alignment nomogram (20), we reduced the hemoglobin concentration by one-third (19) and extrapolated to a summit pH of approximately 7.78.

Our figures predict that, for complete metabolic compensation on the summit, base excess would have to be about -18 meq/l (for blood pH of 7.4). Thus, blood pH is substantially higher than was predicted before the expedition (26). This has important consequences for the interpretation of O_2 transport because of its effect on the OEC, as discussed below (DISCUSSION).

TABLE 2. *Arterial blood measurements at Camp II (6,300 m)*

Subj No.	Gas	pH	P_{CO_2}, Torr	P_{O_2}, Torr	Sat Fraction	BE, meq/l	Tonometry BE, meq/ml
1	Ambient	7.467	19.9	40.0	0.666	−7.2	−6.8
	Hypoxic	7.508					
2	Ambient	7.484	19.6	38.0	0.734	−6.8	−6.2
	Hypoxic	7.500	19.4	29.9	0.578	−6.5	
3	Ambient	7.455	15.8	39.3	0.762	−9.3	−7.2
	Hypoxic	7.533	14.0	25.0	0.457	−8.5	
Mean	Ambient	7.467	18.4	39.1	0.721	−7.9	−6.7
	Hypoxic	7.514	16.8	27.4	0.518	−7.5	

P_{CO_2}, CO_2 partial pressure; P_{O_2}, O_2 partial pressure; Sat, saturation; BE, base excess.

TABLE 3. *Blood from two returning summiters taken at 8,050 m*

Variable	*Subj PH*	*Subj CP*
Measured values		
Hb, g/dl	18.0	18.7
Hct, %	50.0	54.0
RBC × 10⁻⁶/fl	6.40	6.55
2,3-DPG, [DPG]/[Hb]	1.20	1.12
$P_{A_{CO_2}}$, Torr*	13.3	12.5
$P_{A_{O_2}}$, Torr*	31.4	36.3
R*	0.67	0.91
Blood tonometry		
P_{CO_2}, Torr	22.6	22.6
P_{O_2}, Torr	36.6	37.7
pH	7.425	7.460
Saturation, fraction	0.597	0.720
BE, meq/l	−8.4	−5.9
(HCO₃⁻), meq/l	14.4	15.6
Calculated values, South Col		
MCHC, g/dl	36.0	34.6
MCH, pg/cell	28.1	28.5
MCV, fl	78.1	82.4
pH, in vivo†	7.520	7.585
P_{50}, 7.4	31.8	27.4
P_{50}, in vivo	27.7	22.1

RBC, red blood cell; $P_{A_{CO_2}}$, alveolar P_{CO_2}; $P_{A_{O_2}}$, alveolar P_{O_2}; R, respiratory exchange ratio; MCH, mean corpuscular hemoglobin; MCV, mean corpuscular volume. See Table 1 for additional abbreviations. * See Ref. 25. † Assume $P_{A_{CO_2}} = P_{A_{CO_2}}$.

P_{50}. Blood P_{50} values at sea level and Camp II are given in Table 1. The values have been adjusted to pH 7.4 using a Bohr factor of −0.45, corresponding to a 2,3-DPG/Hb ratio of 1.25 (18). As a check of these values, the P_{50}'s estimated from our previous work relating 2,3-DPG, pH, and P_{CO_2} (28) are 28.3 Torr at sea level and 29.4 Torr at Camp II, compared with mean measured values of 28.1 and 29.8 Torr. Thus the P_{50} is completely accounted for by the known effectors of red cell oxygen affinity (2,3-DPG, pH, and P_{CO_2}); no additional factors need be postulated.

The P_{50} values for the South Col blood cannot be taken as representative of the group as a whole, because only two samples were studied. However, P_{50} increased in *CP* between Camp II and the South Col; 2,3-DPG also increased, whereas in pH, the difference was within the limits of the error found in sea level control experiments (±0.08 mol/mol) (27).

Alveolar gas was measured on the summit in only one climber (*CP*). The data and calculations therefrom are given in Table 4. To calculate the summit P_{50} in this subject, we used an in vivo blood pH of 7.78. The subjects P_{50} at pH 7.4 was 27.6 Torr; if the Bohr factor was −0.45, the P_{50} on the summit would have been 19.4 Torr.

Table 5 and Fig. 5 summarize the P_{50} measurements. The P_{50} at pH 7.4 increases with increasing 2,3-DPG up to 6,300 m, but then decreases slightly, even though 2,3-DPG increases further. This may be explained by the lower P_{CO_2} at which measurements were made at 6,300 m compared with sea level and the small number of observations above 6,300 m. However, a precise description of the effect of very low P_{CO_2} on the whole blood OEC is still lacking (28).

DISCUSSION

Hemoglobin and hematocrit. Two previous expeditions to similar altitudes reported hematologic data in sufficient numbers to permit detailed comparisons (3, 14); this is shown in Table 6. Using a *t* test for small samples, we found that our red cell counts at 6,300 m were not different from those in 1973. Our hemoglobin and hematocrit values were slightly different from those in 1960-61 ($P = 0.025$) but clearly different from those in 1973 ($P < 0.005$).

Of the measurements compared, hemoglobin concentration should be the simplest to verify. Unfortunately we were not given the details of the methods for blood collection or measurements in the previous studies. In the 1960-61 expedition, a subsample ($n = 6$) of subjects in whom red cell mass was measured had a mean hemoglobin concentration of 19.1 g/dl (14). These data were from estimates of CO capacity, measured volumetrically. In 1973, the mean hemoglobin concentration for 10 sub-

TABLE 4. *Measurements taken at summit*

	Subj CP
Measured values	
$P_{A_{CO_2}}$, Torr*	7.5
$P_{A_{O_2}}$, Torr*	35
BE, meq/l (from South Col)	−5.9
Calculated values	
$P_{a_{O_2}}$, Torr*	28
P_{50}, pH 7.4, Torr	27.4
pH, in vivo	7.78
P_{50} in vivo, Torr	19.4

$P_{a_{O_2}}$, arterial P_{O_2}. See Tables 1 and 3 for additional abbreviations. * See Ref. 25.

TABLE 5. *Blood O_2 affinity*

Location	pH	n	[DPG]/[Hb]	$P_{a_{CO_2}}$, Torr	$P_{a_{O_2}}$, Torr	P_{50} 7.4, Torr	P_{50} in vivo, Torr	$S_{a_{O_2}}$
Sea level	7.399	18	0.84	40	90	28.1	28.1	0.95
6,300 m	7.467*	14	1.04	18.4†	39.1†	29.8	27.6	0.71
8,050 m	7.552	2	1.18	12.8		29.6	24.9	
8,848 m	7.78	1		7.5	28†	27.4	19.4	0.72

[DPG]/[Hb], 2,3-diphosphoglycerate acid-to-hemoglobin concentration ratio; $P_{a_{CO_2}}$, arterial P_{CO_2}; $P_{a_{O_2}}$, arterial P_{O_2}; P_{50}, P_{O_2} at which hemoglobin is 50% saturated; $S_{a_{O_2}}$, saturation of hemoglobin with O_2 in arterial blood. * Arterial measurements, Table 2. † See Ref. 25.
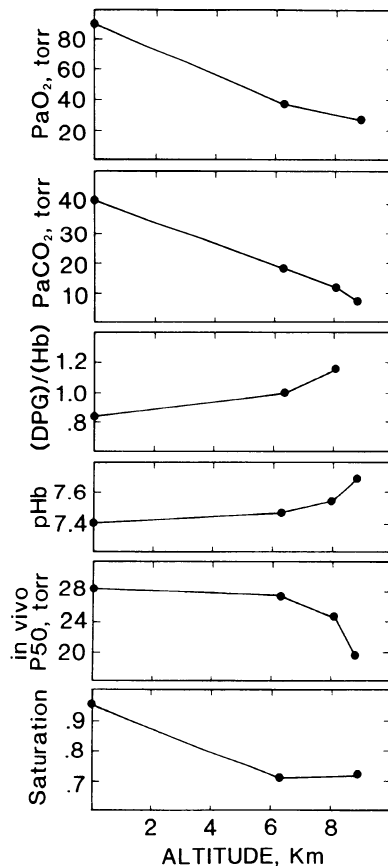
FIG. 5. Effectors of blood O_2 affinity. Data are from Table 5. Increasing blood pH (pH$_b$) decreases PO_2 at which hemoglobin is 50% saturated (P$_{50}$). Net result (*bottom panel*) is that arterial O_2 saturation is protected at extreme altitude. Pa$_{O_2}$, arterial PO_2; Pa$_{CO_2}$, arterial PCO_2; DPG, 2,3-diphosphoglycerate.

TABLE 6. *Hematologic findings at extreme altitude*

Yr	Altitude, m	Hb, g/dl	Hct, %	RBC, ×10⁻⁶/fl	MCV, fl	MCH, pg/cell	MCHC, g/dl	Ref.
1960-61	5,790	19.6±1.0 (24)	55.8±3.3 (21)	5.60±0.43 (18)	99.6	35.0	35.1	14
1973	5,350	20.6±0.4 (10)	63.8±4.6 (10)	6.57±0.68 (10)	97.1	31.4	32.3	2
1981	5,400	17.8±1.0 (20)	50.8±2.6 (20)		80.8	28.4	35.2	
	6,300	18.8±1.5 (19)	53.4±4.0 (18)	6.61±0.40 (18)				

Values are means ± SD for no. of measurements given in parentheses. See Tables 1 and 3 for definitions.

jects at 5,350 m was 20.6 g/dl. These measurements were by the cyanomethemoglobin method, but the instruments, standards, and method of blood collection and storage were not given in the report (3) and some of the subjects had resided at altitudes greater than 5,000 m for extended periods of time (P. Cerretelli, personal communication).

Hematocrit, although more directly related to blood viscosity than hemoglobin concentration, is more difficult to measure accurately and even more difficult to verify. High hematocrits require longer centrifugation times and higher relative centrifugal fields (RCF). The

National Committee for Clinical and Laboratory Standards (NCCLS) (14) defines the RCF

$$RCF = 1.18 \times 10^{-5} \times r \times n^2 \qquad (2)$$

where r is the rotating radius (cm) and n is the rotating speed (rpm). For our centrifuge, RCF = 5,400. According to Pugh (14), their centrifuge had a radius of 10 cm and operated at 3,000 rpm; hence, RCF = 1,062. The centrifuge used in 1973 (3) operated at 3,500 rpm but the rotating radius is not given. In addition to these considerations, the NCCLS recommends that, when hematocrits are greater than 50%, RCF at the top of the blood column should be greater than 3,000. It is also essential to subject the samples to repeated centrifugations to verify that the hematocrit is minimal. It is possible, therefore, that neither of the two previous methods provided a sufficient centrifugal field to properly pack the cells.

Another suggestion that the 1973 hematocrits were too high comes from the distinctly low MCHC's found. Our data and those from 1960-61 agree quite well (35.2 g/dl and 35.1 g/dl), whereas the 1973 data are unusually low (32.3 g/dl). Since the MCHC is the ratio of hemoglobin concentration to hematocrit, this seems best explained by a spuriously high hematocrit.

Mean corpuscular hemoglobin concentration. In spite of their concerted efforts to take in large amounts of liquid, MCHC increased in all expedition members. Since the flow properties of red cells are dependent, to some extent, on MCHC, understanding of this point could be of practical and theoretical importance. Unfortunately, we do not know whether red cell dehydration results from body dehydration or from some abnormality of red cell water transport. For example, it is known that 2,3-DPG influences the Donnan equilibrium of the red cell membrane to alter the relation between intracellular and extracellular pH (18). Changes in pH can affect red cell volume in vitro (29), but in vivo effects have not been demonstrated (1). This problem is in need of further study.

Hemoglobin saturation. A potential difficulty in the method at extreme altitude may be a lack of sufficient O_2 dissolved in the borate buffer to completely oxygenate the hemoglobin when a sample is introduced; that this was not the case can be calculated as follows. If the cuvette volume is 1 ml, temperature is 25°C, barometric pressure is 350 Torr, and the solubility of O_2 in the borate buffer is 1.49×10^{-6} mol·l⁻¹·Torr⁻¹ (15), the total O_2 in the cuvette would be 1.09×10^{-7} mol. If hemoglobin is half-saturated with O_2 and its concentration is 20 g/dl, 0.62×10^{-7} mol of O_2 would be required for conversion of deoxy to oxyhemoglobin. Thus a twofold excess O_2 was present; this is the worst case, because the hemoglobin concentration was usually less than 20 g/dl, and the temperature usually less than 25°C (thereby increasing O_2 solubility). In fact, after addition of hemoglobin to the borate buffer, the mean PO_2 was 52.0 Torr (range 45.5 − 58.3 Torr) for 21 determinations at Camp II. For the same 21 samples, the mean measured O_2 saturation was 0.646 range 0.555 − 0.736). Since hemoglobin can be assumed to be 100% saturated with O_2 at a PO_2 of 30

Torr in the borate buffer (15), our measurements are accurate, even at Camp II.

Blood O₂ equilibrium curve. Shifts in the position and shape in the blood OEC have intrigued physiologists and biochemists for many years because of the implications for O_2 transport and for adaptation to high altitude. Measurements made primarily in Andes of South America suggested that the OEC shifts to the right, thereby augmenting release of O_2 to tissues (10). Most previous results, however, were made under standard conditions of pH and P_{CO_2}, and it has been appreciated only recently that, because of incomplete compensation for respiratory alkalosis, in vivo P_{50} up to 4,250 m is very close to that found at sea level (27).

The importance of the red cell glycolytic intermediate, 2,3-DPG, was first appreciated in 1967 (2, 4). This compound has a strong influence on the oxygenation properties of hemoglobin; it shifts the OEC to the right, a potentially important mechanism for increasing O_2 supply to tissues during anemia. The discovery was important for high altitude work, not only because of this effect on the OEC, but also because it is quickly metabolized in vitro and therefore must be measured in fresh blood samples. Much data from high altitude expeditions must be disregarded because 2,3-DPG was not measured or because samples were not studied while fresh.

A second problem with studies of the blood OEC at high altitude is that many measurements have been made under standard conditions; in vivo pH and P_{CO_2} have usually not been considered. A P_{50} value at pH 7.4, P_{CO_2} = 40 Torr has little relevance for a climber whose P_{CO_2} may be 7.5 Torr and pH nearly 7.8, as in the case of the present study. Before AMREE, no good data were available on arterial conditions at extreme altitude. The studies of Weiskopf and Severinghaus (23) also demonstrated a respiratory alkalosis, but they did not measure 2,3-DPG.

We have tried to measure all of the physiologic parameters of P_{50}. In a previous study, we measured P_{50} and 2,3-DPG in a large number of natives of the Peruvian Andes (27). These measurements, coupled with in vivo pH and P_{CO_2}, allowed us to conclude [as did Weiskopf and Severinghaus (23)] that a small right shift of the OEC, due to increased 2,3-DPG, is offset, in vivo, by a left shift, due to respiratory alkalosis. The net result was that in vivo P_{50} was indistinguishable from sea-level controls.

Blood pH. We previously reported that polycythemia decreases the apparent blood pH at constant plasma pH (27). The mechanism of the effect is not understood, but we believe the most reliable pH would be that of plasma. However, existing nomograms and computer algorithms for blood acid-base and gas calculations use blood pH measured at unspecified hematocrits. Recognizing these limitations, we have reported blood pH values in Tables 1–6. Any error introduced by hematocrit would, however, be small, since the hematocrits found in our subjects did not increase to the levels reported previously for Andean natives. For example, using formulas 2 and 3 of reference 27, the mean pH at Camp II of 7.413 would increase only 0.008 pH units if it were corrected to hematocrit 45%.

2,3-DPG. Concentration of 2,3-DPG within the red cell is believed to be under the general control of pH (12). In our studies, pH increased strikingly above 6,300 m but with little additional increase in 2,3-DPG. Perhaps mean pH is not really increased; it may fall in exercise or during sleep.

Acid-base balance. We found that respiratory alkalosis is never completely compensated. This finding is in agreement with previous observations in Andean natives at 4,250 m (11, 27) or in Sherpas at similar altitude (11). Sojourners also do not compensate, even after several weeks of acclimatization (11). This failure of blood pH to return to sea-level values is still not understood.

Estimations of in vivo base excess and, even more, arterial plasma pH, are subject to considerable uncertainty arising from several sources. First, most nomograms are most accurate around normal conditions (21); the summit conditions are extreme. Second, the nomograms were constructed assuming blood and plasma pH are equivalent; polycythemia has not been accounted for. Third, the buffering effect of plasma has not been calculated in our studies. Increased MCHC suggests dehydration, which would increase plasma protein concentration. Finally, the in vivo pH-P_{CO_2} relationship is well known to differ from that found in vitro (19).

In spite of the above limitations and qualifications, our data indicate that P_{50} is maintained over a very narrow range up to an altitude of 6,300 m. This maintenance is due to the opposing effects of 2,3-DPG and pH (the Bohr effect). Above 6,300 m, the OEC shifts progressively to the left, due to the stronger effect of alkalosis. The various effectors of the OEC are shown in summary in Fig. 5. Although the estimates of in vivo pH are approximations, the clear rise above 6,300 m will certainly mean that arterial O_2 saturation will be protected. Regardless of the mechanism of the extreme alkalosis, it does appear to be beneficial.

Climbers usually elect to acclimatize for 1 or 2 days below the summit, then attempt the summit in sudden pushes. The progressive shift to the left of the OEC above Camp II suggests that this intuitive climbing strategy might be a good one; a left shift could confer some advantage because arterial saturation increases. Indeed, experimental animals survive better at extreme altitude when the OEC is left shifted (5), and persons with mutant hemoglobins with high O_2 affinity apparently acclimatize more efficiently at moderate altitude than their normal counterparts (9).

REFERENCES

1. BEAUMONT, W. VAN, AND R. H. ROCHELLE. Erythrocyte volume is acidified venous blood from exercising limbs. *Pfluegers Arch.* 343: 41–48, 1973.
2. BENESCH, R., AND R. E. BENESCH. The effect of organic phosphates from human erythrocyte on the allosteric properties of hemoglobin. *Biochem. Biophys. Res. Commun.* 26: 659–667, 1967.
3. CERETELLI, P. Limiting factors to oxygen transport on Mount Everest. *J. Appl. Physiol.* 40: 658–667, 1976.
4. CHANUTIN, A., AND R. R. CURNISH. Effect of organic and inorganic phosphates on the oxygen equilibrium curve of human erythrocyte. *Arch. Biochem. Biophys.* 121: 96–102, 1967.
5. EATON, J. W., T. D. SKELTON, AND E. BERGER. Survival at extreme altitude: protective effect of increased hemoglobin-oxygen affinity. *Science* 185: 743–744, 1974.
6. ERSLEV, A. J. Erythroid adaptation to altitude. *Blood Cells* 7: 495–508, 1981.
7. FRASER, C., AND F. MCCALL. A laboratory evaluation of the Compur M1000 Miniphotometer and the Compur M1100 Minicentrifuge. *Clin. Lab. Haematol.* 1: 299–303, 1979.
8. GUYTON, A. C., C. E. JONES, AND T. G. COLEMAN. *Cardiac Output and its Regulation* (2nd ed.). Philadelphia, PA: Saunders, 1973.
9. HEBBEL, R. P., J. W. EATON, R. S. KRONENBERG, E. D. ZANJANI, L. G. MOORE, AND E. M. BERGER. Human llamas. Adaptation to altitude in subjects with high hemoglobin oxygen affinity. *J. Clin. Invest.* 62: 593–600, 1978.
10. HURTADO, A. Animals in high altitudes: resident man. In: *Handbook of Physiology. Adaptation to the Environment.* Washington, DC: Am. Physiol. Soc., 1964, sect. 4, chapt. 54, p. 843–860.
11. LAHIRI, S., AND J. S. MILLEDGE. Acid-base in Sherpa altitude residents ad lowlanders at 4,880 m. *Respir. Physiol.* 2: 323–334, 1967.
12. LENFANT, C., J. D. TORRANCE, AND C. REYNAFARJE. Shift of the O_2-dissociation curve at altitude: mechanism and effect. *J. Appl. Physiol.* 30: 625–631, 1971.
13. National Committee for Clinical Laboratory Standards. *Tentative Standard Procedure for the Determination of Packed Cell Volume by the Microhematocrit Method.* 1: 201–224, 1981.
14. PUGH, L. G. C. E. Blood volume and haemoglobin concentration at altitudes above 18,000 ft (5,500 m). *J. Physiol. London* 170: 344–354, 1964.
15. ROSSI-BERNARDI, L., M. PERRELLA, M. LUZZANA, M. SAMAJA, AND I. RAFFAELE. Simultaneous determination of hemoglobin derivatives, oxygen content, oxygen capacity, and oxygen saturation in 10 μl of whole blood. *Clin. Chem.* 23: 1215–1225, 1977.
16. SAMAJA, M., A. MOSCA, M. LUZZANA, L. ROSSI-BERNARDI, AND R. M. WINSLOW. Equations and nomogram for the relationship of human blood P_{50} with 2,3-diphosphoglycerate, CO_2, and H^+. *Clin. Chem.* 27: 1856–1861, 1981.
17. SAMAJA, M., AND E. ROVIDA. A new method to measure the hemoglobin oxygen saturation by the oxygen electrode. *J. Biochem. Biophys. Methods* 7: 143–152, 1983.
18. SAMAJA, M., AND R. M. WINSLOW. The separate effects of H^+ and 2,3-DPG on the oxygen equilibrium curve of human blood. *Br. J. Haematol.* 41: 373–381, 1979.
19. SIGGARD-ANDERSON, O. An acid-base chart for arterial blood with normal and pathophysiological reference areas. *Scand. J. Clin. Invest.* 27: 239–245, 1971.
20. SIGGAARD-ANDERSON, O., AND K. ENGEL. A new acid-base nomogram. An improved method for the calculation of the relevant blood acid-base data. *Scand. J. Clin. Invest.* 12: 177–186, 1960.
21. THOMAS, L. J. Algorithims for selected blood acid-base and blood calculation. *J. Appl. Physiol.* 33: 154–158, 1972.
22. TUREK, A., AND F. KREUZER. Effect of shifts of the O_2 dissociation curve upon alveolar-arterial O_2 gradients in computer models of the lung with ventilation-perfusion mismatching. *Respir. Physiol.* 45: 133–140, 1981.
23. WEISKOPF, R. B., AND J. W. SEVERINGHAUS. Lack of effect of high altitude on hemoglobin oxygen affinity. *J. Appl. Physiol.* 33: 276–177, 1972.
24. WEST, J. B. Man at extreme altitude. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 52: 1393–1399, 1982.
25. WEST, J. B., P. H. HACKETT, K. H. MARET, J. S. MILLEDGE, R. M. PETERS, C. J. PIZZO, AND R. M. WINSLOW. Pulmonary gas exchange at the summit of Mt. Everest. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 55: 678–687, 1983.
26. WEST, J. B., AND P. D. WAGNER. Predicted gas exchange on the summit of Mt. Everest. *Respir. Physiol.* 42: 1–16, 1981.
27. WINSLOW, R. M., C. C. MONGE, N. J. STATHAM, C. G. GIBSON, S. CHARACHE, J. WHITTEMBURY, O. MORAN, AND R. L. BERGER. Variability of oxygen affinity of blood: human subjects native to high altitude. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 51: 1411–1416, 1981.
28. WINSLOW, R. M., M. SAMAJA, N. J. WINSLOW, L. ROSSI-BERNARDI, AND R. I. SHRAGER. Simulation of the continuous blood O_2 equilibrium curve over the physiologic range of pH, 2,3-diphosphoglycerate, and P_{CO_2}. *J. Appl. Physiol.: Respirat. Environ. Exercise Physiol.* 54: 524–529, 1983.
29. WRANNE, B., R. D. WOODSON, AND J. C. DETTER. Bohr effect: interaction between H^+, CO_2, and 2,3-DPG in fresh and stored blood. *J. Appl. Physiol.* 32: 749–754, 1972.