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Sherpas living permanently at high altitude: A new pattern of adaptation

(hemoglobin/blood/hypoxia/2,3-diphosphoglycerate/Nepal)

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ABSTRACT **Adaptation of Sherpas to high altitude has been studied and compared with that of Caucasians acclimatized to high altitude. Sherpas living permanently at 4000 m above sea level do not have increased hematological parameters (i.e., red cell number, hematocrit, hemoglobin content, and 2,3-diphosphoglycerate/hemoglobin ratio) and have a higher affinity of blood for oxygen as compared with acclimatized Caucasians. Sherpas permanently living at low altitude, on the contrary, have lower affinity of blood for oxygen than do Caucasians living at comparable altitude and are mildly "anemic." Various other red cell biochemical parameters (possibly related to adaptation to altitude) have also been studied in the same population.**

We suggest that Sherpas are genetically better adapted to high altitude than are Amerindians living on the Peruvian highlands, possibly as a consequence of a much more prolonged exposure to such an ecological factor of selection as high altitude.

The populations living for thousands of years at high altitudes may adapt to low oxygen tension with a variety of mechanisms (for a review see ref. 1). In a previous paper (2), we have shown that Amerindians living on the Peruvian highlands show an increased Bohr effect (determined on hemolysates diluted in 0.1 M phosphate buffer) when they are compared with acclimatized Europeans. This fact has been interpreted as an evolutionary adaptation to low oxygen tension that results in an increase in the release of oxygen to the tissues. In contrast, analysis of a Sherpa population, living at the same altitude in the Himalayas, failed to show any difference in the oxygen affinity of diluted hemolysates from that of acclimatized Europeans (3).

In order to understand how Sherpas have achieved their remarkable adaptation to altitude, we have studied in the present research, among a Sherpa population living permanently at 3800–3900 m above sea level, (*i*) some hematological parameters, i.e., red cell count, hematocrit value, and hemoglobin (Hb) content; (*ii*) oxygen dissociation curves determined on whole blood; (*iii*) concentration of some metabolites possibly involved in the adaptation to altitude, i.e., 2,3-diphosphoglycerate (2,3 P_2G), ATP, ADP, lactate, and reduced glutathione; (*iv*) rate of synthesis of 2,3-P_2G in presence and absence of oxygen; and (*v*) activity of some enzymes of the erythrocyte. Most of these parameters were determined also on some Sherpas living permanently at 1200 m above sea level.

This research was carried out in October 1974 in two small Nepalese villages in the Solo-Khumbu region (Kunde, 3800 m and Kumjung, 3900 m above sea level). A few samples were from Namche Bazar (3500 m) and Thami (3900 m above sea level). Red cell counts and hematocrit values were determined in the field within 24 hr after bleeding. Red cell counts were the means of duplicate determinations; they were repeated if the difference between duplicates exceeded 10%.

Hb concentration was determined as cyanmethemoglobin (4). The histograms in Fig. 1 show the individual distribution of the hematological parameters in the two Sherpa populations and in a small group of Caucasians living for 1 month at comparable altitudes. The most relevant fact is that both Sherpa populations have hematological values which are quite similar to those previously determined for Europeans living at sea level and well below the values determined both for Europeans and Amerindians (5) permanently living at the same altitude. Hematological values of the Sherpas living at low altitude (in Kathmandu), are, however, lower than those of the Sherpas living at high altitudes.

The oxygen dissociation curves were determined in whole blood 5–6 days after sampling, at constant pH and CO_2 pressure (6). The data (Fig. 2 and Table 1) show that the oxygen affinity curve in the blood of Sherpas living at high altitudes is strongly shifted toward the left when compared to that of Caucasians living at sea level. The oxygen pressure at which 50% of hemoglobin is saturated, P_{50}, of whole blood of Sherpas living at high altitude is very close to that determined on diluted hemolysates (in 0.1 M phosphate buffer pH 7.4), while in other populations the P_{50} of a hemolysate is always lower than that determined on the whole blood. Apparently the oxygen affinity of the blood of Sherpas at high altitude is not much influenced by the red cell environment. Since only two samples of acclimatized Caucasians were available, statistical methods cannot be used. A shift toward the left caused by acclimatization is, however, surely present in the oxygen dissociation curve of one (Gio.) of the two Caucasian samples; in fact this value, with a 2,3-P_2G/Hb ratio equal to 0.9, would be 23.36, that is, very similar to that of Sherpas.

The Sherpas living at low altitudes (in Kathmandu) have, on the contrary, a curve strongly shifted toward the right, even if compared with that of the Caucasians. The shift is only in part caused by the increase of 2,3-P_2G/Hb ratio.

Red cell metabolites were measured 5 days after sampling in neutralized perchloric acid extracts prepared immediately after bleeding. As shown in Table 2, lactate, total glutathione, ATP, and ADP were greatly increased both in Sherpas living at high altitude and acclimatized Caucasians. 2,3-P_2G concentration in Sherpas living at high altitude is equal to that of Caucasians living at sea level and the concentration of this metabolite is greatly increased in acclimatized

Abbreviations: 2,3-P_2G, 2,3-diphosphoglycerate; Hb, hemoglobin; P_{50}, oxygen pressure at which 50% of the hemoglobin is saturated.

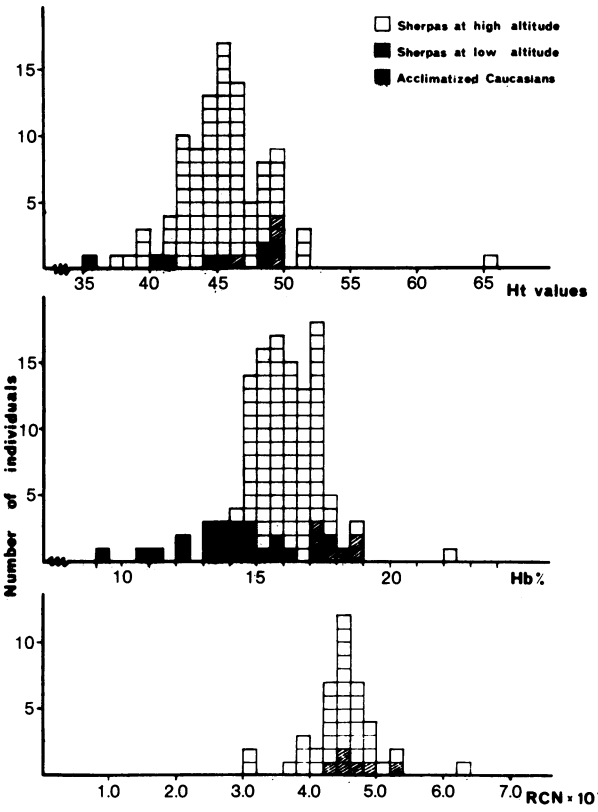

FIG. 1. Hematological parameters of Sherpas at high and low altitude, and of acclimatized Caucasians. Ht, Hematocrit, RCN, red cell count.

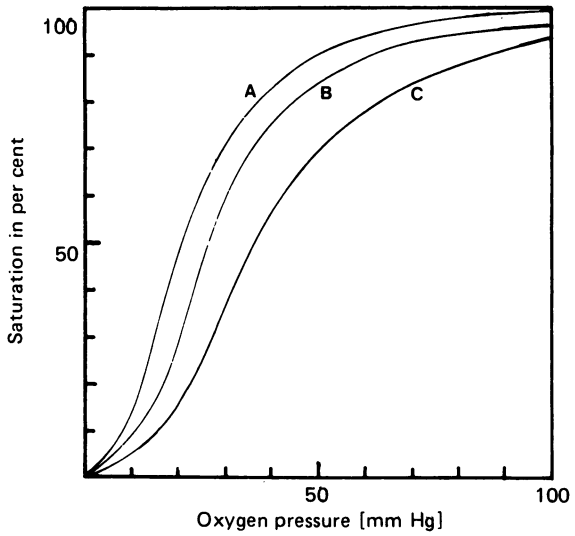

FIG. 2. Oxygen dissociation curves of blood of (A) Sherpas at high altitude; (B) Caucasians at sea level; (C) Sherpas at low altitude (1200 m above sea level). The Hill coefficient (n) calculated on blood of one Sherpa was 2.76.

Caucasians, whereas, the ATP/ADP ratio was almost the same in the three groups. Values for red cell metabolites in the Sherpas of Kathmandu are lacking due to an accident in the transportation of the samples. Previous data show, however, that 2,3-P_2G concentration is increased in the Sherpas of Kathmandu when compared with that of Caucasians living at sea level (3).

The influence of oxygenation and de-oxygenation on 2,3-P_2G synthesis was tested in the presence of inosine, pyruvate, and inorganic phosphate. As shown in Table 3, deoxygenation sharply inhibited the 2,3-P_2G buildup in Sherpas living at high altitude and was without any effect in acclimatized or nonacclimatized Europeans and on the Sherpas living at low altitude (in Kathmandu).

The enzyme activity pattern is shown in Table 4. Hexokinase (HK) and 3-phosphoglycerate kinase (PGK) are significantly increased in both Sherpas and acclimatized Europeans (high altitude Caucasians).

These researches show, in conclusion, that the Sherpa population is better adapted to life at high altitude, at least up to 4000 m, than are Caucasians and Amerindians. This is shown by the fact that Sherpas at 4000 m altitude have normal erythrocyte number, Hb and 2,3-P_2G concentrations whereas both Amerindians and Caucasians react to anoxia with an increase in these parameters (12). It is, however, known that at higher altitude Sherpas also react to anoxia with an increase in red cell number, Hb content, and 2,3-P_2G concentration (3, 13).

Present and previous (3) data show that Sherpas living in Kathmandu have lower Hb levels than do Caucasians living at sea level and Sherpas living at high altitude. The most

Table 1. P_{50} values (mm Hg)* in Sherpas permanently living at high and low altitudes and in high-altitude-acclimatized and nonacclimatized Caucasians

	Sherpas, high altitude	Sherpas, low altitude	Acclimatized Caucasians	Caucasians, low altitude
	22.77	40.86		27.20
	24.35	36.10	(Gio.) 27.86	27.57
	22.77	35.47	(Gui.) 32.03	24.45
	21.50	34.36		29.00
	24.42			
	21.05			
	21.26			
Mean ± SEM	22.59 ± 0.53	36.71 ± 1.43		27.05 ± 0.95
2,3-P_2G/Hb	0.90†	1.36‡	1.26	0.88

* Curves were determined 5 days after bleeding and samples were kept in a refrigerator. In most cases, pH and 2,3-P_2G/Hb were measured at the moment of the oxygen dissociation curve determination. P_{50} values were then corrected by assuming a normal pH of 7.4 and according to the actual 2,3-P_2G, /Hb, following the tables of ref. 7.

† 2,3-P_2G/Hb was determined at the moment of bleeding in four samples. For the other three samples, the mean value of the previous four was used.

‡ This value is that found in the previous expedition (3) on the very same ethnic group living in Kathmandu.

Table 2. Steady-state levels* of some red cell metabolites

	2,3-P_2G	Lactate	GSH	ATP	ADP	ATP/ADP
High altitude						
Sherpas	4983 ± 111.6	1928 ± 145.5	3736 ± 99.0	1854 ± 32.3	274 ± 12.6	6.8
n	17	17	17	17	17	
Acclimatized Caucasians						
Gio.	7170	2220	3010	1850	260	7.4
Gui.	7170	1560	3716	2050	270	
Low altitude						
Caucasians	4295 ± 99.3	917 ± 88.6	2408 ± 58.7	1059 ± 30.3	120 ± 1.73	8.8
n	12	12	19	12	12	

Red cell extracts were made by vigorously shaking 1 ml of blood with 9 ml of perchloric acid (6% wt/vol) immediately after bleeding. Three milliliters of the clear filtrate were mixed with 200 μl of 10 M KOH and 200 mg of triethanolamine-hydrochloride. The pH of the extract was 6.5–7.0. The assays were performed 5 days after blood withdrawal. Lactate, ATP, ADP, and total glutathione (GSH) were assayed according to Bergmeyer (8), and 2,3-P_2G according to Rose and Liebowitz (9).
* nmol of metabolite per ml of red cells or per ml of blood (lactate); mean ± SEM.

likely explanation of the mildly anemic condition of the Sherpas of Kathmandu is that it is caused by the decreased affinity of the blood for oxygen. The decreased affinity would improve the release of oxygen to the tissues, decreasing at the same time erythropoietin production; this, in turn, would produce "anemia." "Anemia" in this context does not have a really pathological meaning and indicates only that the Hb content of Sherpa blood is lower than that of Caucasians living at sea level. This mechanism does operate in individuals with abnormally high 2,3-P_2G/Hb ratio and therefore with decreased affinity for oxygen (14). The hypothesis that the anemia is caused by malnutrition or parasitic infection and not by adaptation to low altitude cannot, however, be entirely ruled out. The scheme of Table 5 summarizes the most significant findings on whole blood in Caucasians, Amerindians, and Sherpas living at high and low altitudes.

Adaptation to low oxygen tension could in principle be obtained either by lowering the blood-oxygen affinity (improving the release of oxygen to the tissues) or by increasing the affinity for oxygen (improving the oxygenation of the blood).

The adaptation of the Sherpas is probably due to the strong shift of the oxygen dissociation curve toward the left, i.e., to increasing the affinity for oxygen and therefore improving blood oxygenation, i.e., to the latter mechanism. If this is true, the evolutionary adaptation of the Sherpas would have followed the same route as did the adaptation of the other mammals living at high altitude such as llama, vicuña, vizcacha, and yak (15, 16), all of which have oxygen dissociation curves shifted toward the left when compared to curves of animals living at low altitudes. It is not yet known how Sherpas (and mammals living at high altitude) discharge efficiently the oxygen to the tissues in spite of the increased oxygen affinity of their blood. An increased Bohr effect [like that discovered in Amerindians (2)] would represent theoretically the most reasonable mechanism. It is difficult, however, at present to say how much of the observed increase in affinity is genetic and how much is physiological. Because the Sherpas living in Kathmandu have an oxygen dissociation curve strongly shifted toward the right, a physiological adaptation following a change in oxygen pressure can evidently take place. It seems (but data are very limited)

Table 3. Influence of oxygenation and deoxygenation on 2,3-P_2G synthesis*

	Sherpas, high altitude		Sherpas, low altitude		Acclimatized Caucasians		Nonacclimatized Caucasians	
	O_2	N_2	O_2	N_2	O_2	N_2	O_2	N_2
	11.55		16.02	13.84	(Gio.) 10.67	12.00	14.00	10.45
	11.56		18.31	14.87	(Gui.) 8.95	9.95	11.51	11.83
	8.35		14.92	15.15			12.72	11.97
	11.98	5.66	15.10	16.99			10.88	11.55
	13.31						10.38	12.65
	12.90							
	9.41	5.96						
	8.44	4.82						
	7.84							
	7.75							
Mean	10.3	5.48	16.09	15.21	9.81	10.98	11.90	11.69

Six-day-old red cells were washed three times with an isotonic buffer (30 mM triethanolamine, 110 mM NaCl, 10 mM K_2HPO_4, pH 7.5) and incubated (hematocrit: 20%) in 75 mM NaCl; 50 mM Na_2HPO_4, 10 mM inosine, 10 mM pyruvate solution, pH 7.4, 38°. Oxygenation and deoxygenation were obtained by gentle bubbling of air or nitrogen into the probe vessels. At the selected times, samples were taken and perchloric acid extracts were made (see Table 2).
* Net 2,3-P_2G production as nmol/ml of red cells per 90 min.

Table 4. Activity* of some red cell enzymes in Sherpas and Caucasians

	HK	PGI	ALD
High altitude Sherpas	55 ± 4.1	1821 ± 89.5	190 ± 13.0
n	10	10	10
Low altitude Sherpas	35 ± 3.4	1619 ± 124.8	207 ± 16.6
n	5	5	5
High altitude Caucasians			
Gio.	74	1988	199
Gui.	75	1990	199
Low altitude Caucasians	26 ± 3.1	1704 ± 83.8	193 ± 5.7
n	5	5	5

* Activities as nmol of substrate turnover/g of Hb per hour (25°). HK, hexokinase (EC 2.7.1.1); PGI, glucosephosphate isomerase (EC 5.3.1.9); ALD, aldolase (EC 4.1.2.13); GAPDH, glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12); PGK, 3-phosphoglycerate kinase (EC 2.7.2.3); PK, pyruvate kinase (EC 2.7.1.40); LDH, lactate dehydrogenase (EC 1.1.1.27); G6PD, glucose-6-phosphate dehydrogenase (EC

that the same capability can be present in some Caucasians too. With normal (0.9) 2,3-P_2G/Hb ratio, the P_{50} of Gio. (Table 1) after acclimatization to high altitude would be 23.36, i.e., very close to that of Sherpas. The P_{50} of Gio. when not acclimatized, with the same 2,3-P_2G/Hb ratio, is 27.65. The data here reported are in agreement with the recent finding of Eaton *et al.* (17) that increased hemoglobin-oxygen affinity increases survival of mice at extreme altitude.

The mechanism of the leftward shift in the oxygen dissociation curve is not clear; the presence of abnormal hemoglobin is to be excluded, since the curves obtained with diluted hemolysates are identical in Sherpa and Caucasian blood. Moreover, the electrophoretic patterns of European and Sherpa hemoglobin are indistinguishable (3). The hypothesis of an allosteric effector cannot be completely ruled out because the oxygen dissociation curves of the hemolysates (3) were done some days after hemolysis and the possible effector could be labile. Similar changes in affinity in the whole blood and not in the hemolysates after adaptation were described by Grigg (18) in the fish *Ictalurus nebulosus*.

Unknown is also the role, if any, of glutathione in this process, but the large increase of this metabolite with increase in altitude should be investigated.

The increases in glycolytic enzyme activity and in lactate, ATP, and ADP levels are clearly nongenetic, since they were observed both in Sherpas and acclimatized Europeans. The only peculiarities of Sherpa erythrocytes are the normal 2,3-P_2G steady-state level and a decreased 2,3-P_2G buildup upon incubation with inosine, pyruvate, and inorganic phosphate in the absence of oxygen. The explanation of these effects, which cooperate in maintaining the leftward shift in the oxygen dissociation curve, is unknown. A possible mechanism could be a lower permeability to inosine in fully deoxygenated Sherpa red cells; nucleoside permeability into the red cell is under genetic control and can vary over a great range according to species (19). The increases in lactate and ATP in the acclimatized groups correlate with the rise in hexokinase and 3-phosphoglycerate kinase activity. It has been shown (20) that glycolytic flux depends on hexokinase activity, whereas, the ATP level is regulated, among other factors, by the 3-phosphoglycerate kinase activity.

Why has the evolutionary adaptation of the Amerindians and Sherpas followed different routes? It must be remembered that man entered America through the Bering strait probably only 35,000 years ago. Amerindians have lived on the Peruvian highlands for more or less 14,000 years (21). That means that they migrated to the highlands very soon after their arrival in South America. Although this is a considerable time it is not sufficient for the evolution in a relatively small population (as it was some thousands of years ago) of very efficient mechanisms of adaptation. Amerindians are poorly adapted to hypoxia as is shown by the pronounced polycythemia and also by the frequent occurrence of "Monge" disease and pulmonary edema. However, Sherpas arrived in their present settlements a few centuries ago after coming from Tibet (also 4000 m above sea level), but we do not know when their ancestors reached Tibet. We do know, however, that human beings have inhabited central Asia for at least 500,000 years and primitive populations of hunters presumably reached the highlands very soon after, as happened in South America. If this is true, the ancestors of the present Sherpas lived at high altitude for several hundred thousand years and had sufficient time to evolve the same efficient mechanism of adaptation to hypoxia as did the other mammals living at comparable altitudes.

Table 5. Data on the physiological parameters of whole blood that are related to oxygen transport

	Hb	Oxygen affinity	2,3-P_2G
Caucasians, low	N	N	N
Caucasians, high	↑	↓	↑
Amerindians, low	N	N	N
Amerindians, high	↑	↓	↑
Sherpas, low	↓	↓	↑
Sherpas, high	N	↑	N
Sherpas, very high	↑	—	↑

N = normal; ↑ = above normal; ↓ = below normal; —, not done.

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Table 4. (*Continued*)

GAPDH	PGK	PK	LDH	G6PD	GR
6863 ± 543	13,350 ± 525	439 ± 33	5434 ± 367	310 ± 23	439 ± 18
10	10	10	10	10	8
5200 ± 419	12,148 ± 395	383 ± 30	5001 ± 165	243 ± 29	505 ± 85
5	5	5	5	5	5
6732	13,760	352	6451	413	254
7583	12,890	512	6029	184	—
5103 ± 346	8208 ± 471	474 ± 12.9	4701 ± 64.5	397 ± 29.4	442 ± 17.7
5	5	5	5	5	5

1.1.1.49); GR, glutathione reductase (EC 1.6.4.2). Heparinized whole blood was kept at 3–4° until tested. The hemolysates [made according to Pescarmona *et al.* (10)] were made 7 days after blood withdrawal. HK, ALD, GAPDH, PK, and LDH were tested according to Pescarmona *et al.* (10); PGI, G6PD, and GR were assayed according to Beutler (11).

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