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Hemoglobin Levels in Persons of Tibetan Ancestry Living at High Altitude (38952)

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(Introduced by F. B. Bang)

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It has been generally held that mean hemoglobin (Hb) levels are identical in normal man living at sea level regardless of geographic and racial differences (1–3), and that this pertains to increments of Hb increase as man adapts to high altitude (2, 3). However, one of the authors (LJS), formerly physician-in-charge of the Kunde hospital located near Mt. Everest, noted that Hb levels of Sherpa patients examined using the Sahli acid-hematin technique did not reveal the expectedly high Hb values usually found at that altitude (4000 m). We subsequently reported (4) that Hb levels of inhabitants of Lang-Tang village, located in northern Nepal (altitude 3700 m, latitude 28° north), were lower than expected, despite no evidence of vitamin B_{12}, folate, or iron deficiencies. The present study pursues this observation in greater detail.

Subjects and Methods. The survey was performed in a different region of Nepal where the Sherpa population, living at 4000 m, is culturally different but genetically similar to the villagers of Lang-Tang, both being of pure Tibetan ancestry. In contrast to Lang-Tang, the Sherpas have available modern medical care, and endemic goiter and hypothyroidism, previously common in both areas, have been virtually eradicated in the Sherpas by use of depot iodine injections (5). Twenty-eight males (mean age 30.0 yr, range 15–57) and 23 females (mean age 35.0 yr, range 15–51) were studied.

Subjects were screened initially by physical examination. If no significant illness was found, the following tests were performed locally; erythrocyte sedimentation rate, urine testing,[2] stool for occult blood, and blood urea nitrogen.[2] If these were normal, serum, EDTA anticoagulated blood, and fresh peripheral blood smears were promptly sent by air to Kathmandu where hematologic studies were begun.

The following tests were performed: microhematocrit, Hb determination (cyanmethemoglobin method, with careful spectrophotometer calibration using a didymium filter and Hycel[3] Hb standards), calculation of the mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, and a Hb H preparation using brilliant cresyl blue, cellulose acetate Hb electrophoresis using TRIS–borate buffer, pH 8.6, and quantitation of Hb A_2 (6) and alkali resistant Hb (7). Serum was frozen for subsequent assays of vitamin B_{12} (8), serum and red cell folate (9), serum iron and iron binding capacity (10), and triiodothyronine and thyroxin levels.[4] These assays were kindly performed by the Pathology Laboratory and Department of Nuclear Medicine at Christchurch Hospital, Christchurch, New Zealand. On four subjects, serum protein, calcium, and magnesium were determined. Evans blue plasma volume estimations were performed on four Sherpas in Kunde, four Americans in Kathmandu (altitude 1300 m) and the authors. All were males in good physical condition. Finally, two normal males of Tibetan ancestry in Lang-Tang were studied at 3700 m for levels of red cell 2,3-diphosphoglycerate (DPG), adenosine triphosphate (ATP), whole blood lactate, and serum inorganic phosphorus.

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[2] Labstix and Azostix, Ames Company, Inc., Elkhart, Indiana.

[3] Hycel, Inc., Houston, Texas.

[4] Immunoassay techniques to be reported.

These assays were performed at the Chelsea Naval Blood Research Laboratory, Chelsea, Massachusetts, on perchloric acid extracts that were made at high altitude according to that laboratory's standard technique (11) and promptly frozen at $-20°$ and shipped to the United States within 10 days.

Because of somewhat difficult field conditions, certain determinations were not performed on all subjects because of delay in transporting specimens or an inadequate volume of serum.

Results. The mean Hb levels were 17.0 $\pm$ 1.25 g/100 ml for males and 15.3 $\pm$ 0.8 g/100 ml for females. Serum vitamin B_{12} and red cell folate were normal in all subjects. Serum triiodothyronine was normal in 39 persons tested. Four of 35 subjects had low free thyroxin, but there was no correlation with Hb levels. Serum iron determinations were performed on 41 subjects. Nine (22%) had either a serum iron less than 55 μg% or a transferrin saturation less than 15%. When only subjects known to be normal were evaluated, the mean Hb for males was 17.1 $\pm$ 1.1 g/100 ml and for females 15.3 $\pm$ 0.6 g/100 ml. The mean Hb in subjects in which either serum iron or MCHC were not obtained was 17.1 $\pm$ 1.4 g/100 ml for males and 15.5 $\pm$ 1.0 g/100 ml for females; the mean Hb for five males and four females with a low serum iron, percentage of transferrin saturation or MCHC was 16.8 g/100 ml and 14.9 g/100 ml, respectively. None of these subgroup values differ significantly from the total population or from each other.

The mean Hb A_2 was 2.96 $\pm$ 0.58%, with only one subject being slightly elevated (4.7%). The mean fetal Hb was 0.9 $\pm$ 0.3%, one subject being slightly elevated (2.06%). No other abnormalities were found in these two persons. Reticulocyte counts on 38 subjects were all normal and Hb H preparations on the same persons were negative. One subject was a Hb E heterozygote (28% Hb E). All other electrophoreses were normal.

Four persons studied had normal total serum protein, calcium and magnesium. Plasma volume determinations in four Sherpas studied at high altitude were identical to those obtained in four Americans in Kathmandu and the authors when expressed as cc/kg body wt. No association between ABO blood group and Hb level was found.

The results of assays of DPG, ATP, whole blood lactate, and serum inorganic phosphate on two normal subjects of Tibetan descent in the village of Lang-Tang are shown in Table I. Organic phosphate levels were somewhat lower than those found in normal persons at sea level whose blood was processed under usual laboratory conditions, rather than those encountered in Lang-Tang.

Discussion. The expected mean Hb for males living at 4000 m is at least 19 g/100 ml, with females correspondingly lower (2, 12). However, the mean Hb of Sherpas was similar to that found in Lang-Tang where the values for males and females were 16.8 $\pm$ 1.4 g/100 ml and 14.5 $\pm$ 0.7 g/100 ml, respectively (4). Plasma volume determinations indicate that the low Hb levels observed were not the result of an inordinately large plasma volume. The study thus confirms that persons of Tibetan

TABLE I. ERYTHROCYTE 2,3-DIPHOSPHOGLYCERATE (DPG) AND ADENOSINE TRIPHOSPHATE (ATP) IN TWO NORMAL MALES OF TIBETAN ANCESTRY LIVING AT 3700 m[a].

	Hb g/100 ml	MCHC	DPG[b]	ATP[b]	Whole blood lactate[b]	Serum creatinine mg/100 ml	Serum inorganic phosphorus mg/100 ml
Subject 1	14.4	33.7%	10.29	3.48	0.62	0.8	5.4
Subject 2	16.6	33.3%	10.06	3.34	1.14	0.8	5.9

[a] These data have been previously reported (*Proc. Soc. Exp. Biol. Med.* 148, 701, 1975) for use as control values.

[b] Normal values: 2,3-DPG: 12 $\pm$ 1.2 μM/g Hb; ATP: 4.5 $\pm$ 0.4 μM/g Hb; Lactate: less than 1.0 μM/ml whole blood on fresh specimens.

ancestry in this area have a uniquely low red cell mass with respect to altitude.

The low Hb levels were not explained by (1) age (13); (2) deficiencies of vitamin B_{12}, folate, iron, or thyroid hormone; (3) systemic disease; or (4) hemoglobinopathies. Perhaps deficiency of another hematinic exists in the Himalayas, but adaptive or evolutionary physiologic changes may be responsible for our findings. An increase in total blood volume is reportedly a major adaptive mechanism of man to high altitude (14, 15). However, indirect estimation of total blood volume, calculated from the plasma volume and hematocrit and related to body weight, suggests our subjects rely little on this. Data on cardiac output support the concept that greater O_2 extraction is more important than increased blood flow in supplying the body's O_2 requirements at high altitude (16). In this regard hyperventilation is important (17), as it increases arterial O_2 saturation. Detailed studies of O_2 transport were not performed, and it remains possible that Sherpas have unique ventilatory efficiency. However, increased pulmonary diffusing capacity has been documented in Andeans (18) despite their higher Hb levels and not uncommon chronic mountain sickness.

If our Hb data are superimposed on the compiled data of Hurtado *et al.* (2) (Fig. 1), it can be seen that the mean Hb of males in the present study corresponds to that expected at 2300 m and an arterial O_2 saturation of 92% rather than the expected O_2 saturation at 4000 m (approx 84%). This might be explained by a very low Hb O_2 affinity which permits easy release of O_2 to the tissues, thereby avoiding hypoxic stimulus to erythropoietin production and subsequent increase in red cell mass. Interestingly, however, chronic mountain sickness has not been recorded among the Sherpas. This syndrome may be the result of overcompensation of the DPG mechanism, as extreme elevations of DPG may decrease O_2 affinity to such an extent that inefficient extraction of O_2 from the air at high altitude results in marked polycythemia (12). An alternative method of adaptation may be increased O_2 affinity,

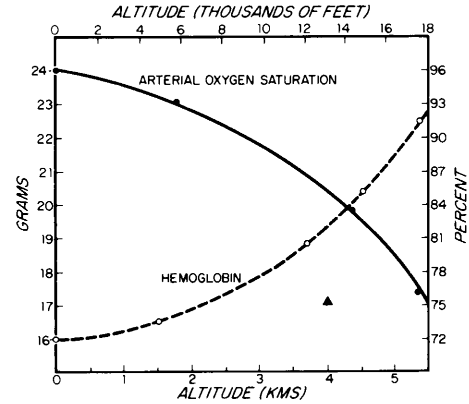

FIG. 1. Relationship of arterial O_2 saturation and mean Hb level to altitude (from *Arch. Internal Med.* **75**, 284, 1945; copyright 1945, American Medical Association). The solid triangle indicates the mean Hb level found in males in the present study. Note that this corresponds to that expected at approximately 2300 m and an arterial saturation of 92%.

which would provide more efficient O_2 extraction from the air. There was no electrophoretic evidence that the predominant Hb of Sherpas differs from Hb A, and Hb F was not increased. Thus mechanisms which alter Hb O_2 affinity should be evaluated. Our data on DPG and ATP are limited and need to be confirmed, but the low levels found are not explained by mean corpuscular Hb concentration or serum inorganic phosphorus. Perhaps the observed Hb levels are consistent with increased O_2 affinity if other physiologic mechanisms, e.g., increased muscle myoglobin concentration (19), increased tissue vascularity (20), and metabolic changes (21, 22), permit adequate tissue oxygenation.

Summary. The mean hemoglobin level of persons of Tibetan ancestry living at 4000 m in the Nepal Himalayas was found similar to that expected at an altitude of 2300 m and an arterial O_2 saturation of 92%. Plasma volumes were normal, and deficiency states and hemoglobinopathies were not significant. Possible reasons for this finding are discussed.

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