Hemoglobin levels, vitamin B_{12}, and folate status in a Himalayan village[1,2]

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The difficulty of access in the high Himalayas of Nepal has hindered understanding of potential nutritional problems that may beset the inhabitants of this area. The large number of persons who live at high altitudes throughout the world and the necessity for research in such areas has recently been emphasized (1). A preliminary survey for hematologic abnormalities carried out in April 1972 by one author (WHA) among the Sherpas living at 13,000 feet near the base of Mt. Everest suggested deficiencies of either folic acid or vitamin B_{12}. This was surmised from fresh peripheral blood smears from 67 subjects, mostly school-age children, of whom 25% had definite polymorphonuclear hypersegmentation. To determine the significance of this finding, the following study was undertaken.

Subjects and methods

In October 1972, blood specimens were collected from 52 adult inhabitants of Tibetan ancestry in the Himalayan village of Lang-Tang, altitude 12,000 feet, located approximately 60 miles north of the capital of Nepal, Kathmandu. This number of specimens included almost 20% of the village population. The time of this study was during the main harvest season in the Himalayas.

Non-anticoagulated Vacutainers were used to collect serum for B_{12} and folate assays. ETDA anticoagulated specimens were used for determinations of hemoglobin, hematocrit, mean corpuscular hemoglobin concentration (MCHC), reticulocyte count, and to prepare hemolysates for red cell folate levels. *Euglena gracilis* and *Lactobacillus casei* were used for bioassays of B_{12} and folate, respectively, with red cell hemolysates for folate assay from 45 subjects prepared according to the method of Hoffbrand (2). In addition, milk samples from the yak, a bovine capable of living at extremely high altitude, were frozen and similarily assayed, the B_{12} assay being performed with and without papain digestion.

Results

The vitamin B_{12} and folate values obtained from humans are presented in Table 1. Only one subject, a 25-year-old female with a hemoglobin of 12.75 g/100 ml (#45) had a significantly low B_{12}. Information as to whether she was pregnant (a cause of low serum B_{12}) was not obtained. This subject also had a low MCHC. All serum folates were normal except for three whose levels were between 5 and 6 ng/ml (an indeterminant range (3)). Six subjects had red cell folates below 150 ng/ml, the lowest being 116 ng/ml. However, all but one of these had normal serum folate levels. The B_{12} and folate content of yak milk are presented in Table 2.

Mean hemoglobin values and one standard deviation for adult males were 16.8 ± 1.4 g/100 ml and for adult females 14.5 ± 0.7 g/100 ml. Subjects with a low MCHC or anemia as defined by the World Health Organization at sea level (3) were excluded from these calculations. Only three (6%) of 52 subjects had an MCHC below 32%. Except for one subject with severe hypochromic anemia, reticulocyte counts were normal. Hemoglobinopathies and thalassemia were not encountered (4).

Discussion

The staple foods in this village included potatoes, buckwheat, and green vegetables such as mustard greens, radishes, and nettles. Potatoes were boiled and skinned before eating. Buckwheat flour is cooked in a variety of methods, occasionally with the addition of hot peppers. The common alcoholic beverage,

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"chang," is prepared from fermented grains, and is drunk by all except the very young. Chicken, mutton, or yak meat are an uncommon but welcome addition to the diet. However, the value of these animals as a source of eggs, wool, and milk usually prevent them from being slaughtered for meat. In addition, there is an element of Buddhist cultural bias against killing of animals, although these people were not vegetarians. Milk availability fluctuates seasonally, and because of the economic advantage in selling milk to a local cheese factory for export, further curtailment of milk supply to the villagers probably occurs.

TABLE 1
Vitamin B_{12} and folate levels in Lang-Tang Village, October 1972

Subject no.	Serum B_{12}, pg/ml	Serum folate, ng/ml	Erythrocyte folate ng/ml
1	640	14.0	300
2	600	9.4	
3	600	16.5	
6	480	6.3	304
9	576	12.2	289
13	220	8.1	235
16	320	9.1	310
17	160	6.2	
18	344	5.6	206
19	240	13.8	289
20	152	7.0	182
21	416	8.4	354
22	320	8.8	
23	320	12.3	216
24	400	16.5	
25	452	8.0	
26	200	13.8	365
27	172	20.5	263
28	400	10.0	245
29	580	11.0	202
31	172	8.1	277
32	360	12.2	265
34	552	10.0	337
35	416	12.2	282
36	480	11.3	328
37	416	13.8	527
38	232	10.0	219
39	1,840	20.0	320
41	520	10.5	149
42	520	9.1	414
43	320	12.2	
44	360	15.0	324
45	128	8.4	163
46	172	11.3	180
48	288	40.0	240
49	430	12.3	342
51	320	15.0	217
52	328	9.0	119
53	208	10.2	297
54	552	7.5	328
55	344	6.7	182
56	504	5.2	145
57	760	7.1	116
58	500	7.6	294
59	300	8.1	160
60	600	7.6	242
61	552	6.7	153
62	560	10.2	177
63	452	8.4	123
64	580	7.5	250
65	552	6.4	126
66	416	5.6	183

TABLE 2
Vitamin B_{12} and folate content of yak milk

	Amount
Folate	31 μg/liter
B_{12}, plain	6,870 pg/ml
B_{12}, papain digested	7,270 pg/ml

The B_{12} content of yak milk (although the correct terminology for a female yak is "nak") is similar to cow's milk; i.e., approximately 6,000 pg/ml (5). However, the folate content was only 31 μg/liter, as compared with almost 55 μg/liter for fresh cow's milk (6). As all milk consumed by the villagers was boiled before drinking, this undoubtedly decreased the folate content further. The butterfat content, on the other hand, is 7 to 9% (7).

Despite the apparently restricted dietary status of the villagers studied, there appeared to be no evidence for significant B_{12} or folate deficiency at the time of this study. Thus, the authors' preliminary observation of neutrophile hypersegmentation noted among inhabitants living near Mt. Everest that suggested megaloblastosis was not substantiated by specific B_{12} and folate assays in a similar high altitude village. However, the former observation was made in April and the latter in October. Thus, the possibility of significant seasonal variation in availability of B_{12} and folate, especially the latter, remains to be evaluated. Nevertheless, it seems unlikely that seasonal deficiency would be an etiologic factor causing anemia in this population. Serum B_{12} levels were, with one exception, normal. The liver stores of vitamin B_{12} are considerable, and their depletion, by virtue of enterohepatic circulation (8), is slow. Indeed, the statement has been made that megaloblastic anemias due to "uncomplicated dietary deficiency of vitamin B_{12} have yet to be demonstrated in man" (9). Similarily, red cell folate levels, an indicator of tissue folate

stores, did not suggest a significant relationship to anemia, as 133 days of almost total dietary deficiency of folate are required to produce early megaloblastic changes in a normal subject (10).

Nevertheless, hemoglobin levels were considerably lower than one would expect for subjects living at that altitude (11, 12). However, only 6% had a low MCHC, indicating a minimal problem with iron deficiency, and a generally adequate level of nutrition of the population is supported by simultaneous anthropological (height versus weight) measurements made of approximately 20 young children in the same village (H. Zeigler, unpublished observations). The only gross deficiency found, and one in common to mountainous areas of Nepal in general, was that of iodine. Goiters were ubiquitous, and clinical hypothyroidism, deaf mutism, and cretinism were occasionally noted. Possibly subclinical hypothyroidism may have some bearing on the lower hemoglobin levels, but further investigation is required. It is possible that genetic factors are involved. If subsequent evidence suggests this is the case, then one must question whether "normal" hemoglobin values for man can be universally applicable, even when one considers the effect of altitude.

Summary

A survey of 52 adult inhabitants of Tibetan ancestry in a village located at 12,000 feet in the Nepal Himalayas revealed lower hemoglobin levels than that reported for populations living at similar altitude elsewhere in the world. However, serum B_{12}, serum and red cell folate, and mean corpuscular hemoglobin concentrations gave no evidence for megaloblastosis or iron deficiency as a cause of anemia. Although the role of other deficiencies such as iodine remains to be evaluated, present data do not suggest that the lower hemoglobin values found are indicative of anemia in this population.

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