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# Hematologic Observations on the Yak (38613)

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(Introduced by F. B. Bang)

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The yak, *Bos grunniens*, belongs to the same genus as the cow, but is unique to that genus in that it survives for long periods at altitudes of up to 6000 m. We herein report hematologic observations on a yak studied at both high and low altitudes over a period of eight months to obtain data relevant to mammalian survival at high altitude.

*Subject and Methods.* The study animal was a one year old male weighing 50 kg when first studied at 4000 m in the village of Lang-Tang in north Nepal. After baseline hematologic data were obtained it was partially exsanguinated by removal of 2500 $cm^3$ of blood over a 4-hr period. It and a control yak were then studied, as detailed below, to observe the response to acute blood loss. Two months later the study yak was flown to Kathmandu (1450 m). Eighteen days after arrival severe hemolytic anemia occurred secondary to babesiosis, and its hemoglobin (Hb) dropped from 10 to 5 g/100 ml. Two similar hemolytic episodes occurred during the next 3 mo, the drop in Hb in all instances being close to that produced by bleeding at high altitude (approximately 50% of the red cell mass). Hematologic studies were performed frequently during this time, but no therapy was given.

Blood was collected in EDTA via an 18 gauge needle placed in the jugular vein. Routine blood counts, Hb concentrations and microhematocrits were measured by standard techniques (1). Platelets were counted by phase microscopy (2). Thrice washed red cells were hemolyzed with water and carbon tetrachloride, and electrophoresis was performed using acrylamide gel in Tris buffer, pH 8.9 (3); cellulose acetate with Tris buffer, pH 8.6; citrate agar, pH 6.0 (4); and starch gel with 0.005 *M* phosphate buffer, pH 7.1 (5) and EBT buffer, pH 8.6 (6). Starch gels were stained with either amido black or benzidine. Hb bands were quantitated by elution from cellulose acetate (7). Globin chains were separated by the method of Garrick *et al.* (8), and quantitated by elution in 0.1 *N* NaOH after staining with Ponceau S. Erythrocyte 2,3-diphosphoglycerate (2,3-DPG), adenosine triphosphate (ATP) and whole blood lactate were measured at the Chelsea Naval Blood Research Laboratory, Chelsea, MA, on perchloric acid extracts which had been prepared according to that Laboratory's standard procedure (9), frozen at high altitude, and shipped to the United States. Other procedures included determination of alkali resistant Hb (10); brilliant cresyl blue (BCB) reduction for glucose-6-phosphate dehydrogenase (G-6-P D) activity (11); and resistance of Hb to heat denaturation (12). Sickle preparations were made with 2% sodium metabisulfite for 24 hr, unstable Hbs were sought by incubating red cells with BCB at room temperature for 4 hr, and new methylene blue was used to stain reticulocytes. Controls for 2,3-DPG, ATP and lactate were two normal humans living at the same altitude. Hb electrophoretic comparisons are given in Fig. 1. The same controls, with the exception of the yak/Jersey cross, were compared for G-6-PD activity, sickling, inclusion body formation, and alkali resistant Hb.

*Results.* The leukocyte count of the study yak was 8–10,000/$mm^3$, with 35% neutrophiles, 55% small lymphocytes, 6% large lymphocytes, 4% monocytes, 1–2% eosinophiles, and rare basophiles. The platelet count was approximately 700,000/$mm^3$. The initial Hb level at 4,000 m was 13 g/100 ml and similar values were obtained on two other yaks. Red cell indices included an MCV of 43 $\mu m^3$, and MCH of 16.2 pg, and an MCHC of 37%.

Figure 1 shows the starch gel electropho-

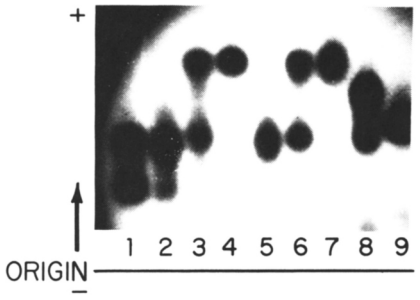


FIG. 1. Electrophoretic comparison of yak Hb to related species, the Asiatic buffalo and human Hb A. Code: 1-yak; 2-yak/Jersey cross ($F_1$); 3-yak/Jersey/zebu cross ($F_2$); 4-zebu (Asiatic cow); 5-Jersey cow; 6-zebu/Jersey cross; 7-zebu; 8-Asiatic buffalo; 9-human Hb A. Note the diminishing proportion of the slow band (*Hb slow*) of the yak in $F_1$ and $F_2$ generation non-yak crosses. Starch gel, EBT buffer, pH 8.6, benzidine stain.

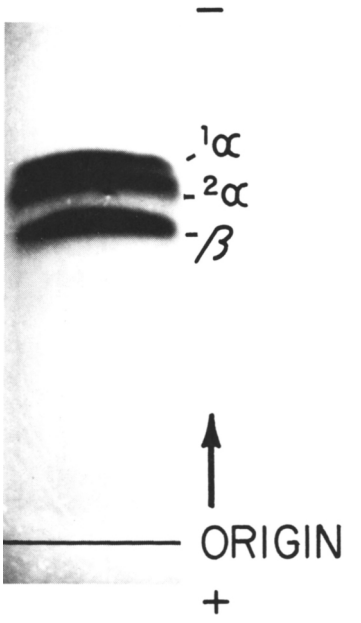


FIG. 2. Globin chains of yak Hb. The ratio of $\beta$ to $^1\alpha + {}^2\alpha$ was 1:1. The ratio of $^1\alpha$–$^2\alpha$ was 37.5:62.5, similar to the ratio of the yak's two Hbs. Cellulose acetate, amido black stain.

resis of yak Hb as compared to various controls. The same pattern was observed on acrylamide gel. The ratio of the slow to fast band (hereafter referred to as *Hb slow* and *Hb fast*) after quantitation from cellulose acetate on 31 occasions over 8 mo averaged 38:62, the % *Hb slow* being 37.8 ± 1.2 (1 SD). This ratio was observed in four other yaks. The proportion of *Hb slow* progressively decreased in $F_1$ and $F_2$ generation non-yak crosses (i.e., 37.8%, 20.0% and 9.1%; see Fig. 1). On agar gel, pH 6.0, only a single broad band was observed on serial specimens, and this migrated cathodally faster than human Hb A. Three globin chains were identified (Fig. 2). The ratio of the slowest band to the two faster bands was 1:1, and the ratio of the fastest to the middle band was 37.5:62.5, similar to the ratio of the two types of Hbs.

The slow Hb band of the yak turned brown within 24 hr after acrylamide gel electrophoresis, suggesting *Hb slow* was more prone to methemoglobin formation. However, elution of the two Hbs from cellulose acetate and allowing them to stand for up to 2 wk at room temperature did not reveal a difference in absorbance at 630 nm. Yak Hb was 100% resistant to the standard human alkali denaturation technique, as was true of the Hbs of the other ruminants studied.

Serial starch gel electrophoreses at pH 8.6 and 7.1 did not give any evidence for production of a new Hb in response to anemia produced by bleeding at high altitude or hemolysis at low altitude. Likewise, prolonged exposure to higher oxygen tensions of low altitude did not cause a preferential decrease in production of either Hb. During recovery from hemolytic anemia, red cells increased in size, the MCV and MCH becoming 54 $\mu m^3$ and 21 pg, respectively, with the MCHC being slightly decreased. Reticulocytes detectable with new methylene blue were not present on routine smears, nor were they found within 39 hr after acute blood loss. However, after the first episode of severe hemolysis, a reticulocytosis was noted on days 5–7. The most striking change produced by phlebotomy was a rapid "plasma shift", in that the Hb concentration reached a nadir within 16 hr. After bleeding the yak's heart rate rose from approximately 50 to 75 beats per min and the respiratory rate remained unchanged.

Serial measurements of red cell 2,3-DPG, ATP and whole blood lactate following partial exsanguination are presented in Table

TABLE I. 2,3-DPG, ATP AND WHOLE BLOOD LACTATE LEVELS IN A PARTIALLY EXSANGUINATED YAK COMPARED TO A NORMAL YAK AND HUMAN CONTROLS AT HIGH ALTITUDE.

| | Time | Hb[a] g% | 2,3-DPG[a] $\mu M$/g Hb | ATP[a] $\mu M$/g Hb | Lactate $\mu M$/cc blood |
|---|---|---|---|---|---|
| Study | Control | 13.0 | 0.04 | 1.08 | 2.56 |
| Yak | 16 hr[b] | 7.25 | 0.09 | 1.34 | 2.20 |
| | 27 hr | 7.50 | 0.05 | 1.64 | 1.70 |
| | 39 hr | 7.25 | 0.08 | 1.35 | — |
| Control | Control | 13.0 | 0.04 | 1.14 | 2.60 |
| Yak | 16 hr | 13.0 | 0.04 | 1.16 | 2.27 |
| | 27 hr | 12.4 | 0.11 | 1.19 | 2.17 |
| | 39 hr | 12.3 | 0.08 | 1.12 | — |
| Human No. 1 | | 14.4 | 10.3 | 3.43 | 0.62 |
| Human No. 2 | | 16.6 | 10.1 | 3.86 | 1.14 |

[a] Abbreviations explained in text.
[b] Hours after bleeding.

I. The baseline values of 2,3-DPG were extremely low, and did not increase during the 39 hr after bleeding as compared to the control yak. ATP levels were lower than those found in man, and there was only a slight rise in ATP after bleeding. Control studies performed simultaneously on humans gave close to normal values, suggesting that the low values found in the yak were not artifacts produced by technical procedures performed in the field.

The BCB dye reduction test showed the Asiatic buffalo reduced the dye at the same rate as normal human controls. However, all other animals, which are in a genus distinct from the buffalo, gave a delayed response. The yak was by far the most inefficient of all animals studied in reducing BCB, taking twice the time of human controls. Because of this observation, Heinz body preparations were performed during hemolytic episodes to see if there was evidence for chemically induced hemolysis which might have followed ingestion of certain plants. No Heinz bodies were found, nor were they noted after incubation of routine specimens with BCB. The heat denaturation test was negative.

Yak red cells did not sickle on exposure to sodium metabisulfite or on standing at room temperature or 4° for 24 hr. Crenation occurred more rapidly in anticoagulated blood when compared to humans and the other animals studied, and this was found with EDTA, balanced oxalate, ACD and heparinized specimens. However, immediate examination of a wet preparation under a glass coverslip revealed less than 1% of cells to be crenated. The normal shape was expectedly circular and biconcave. The effect of pH on cell shape was not investigated.

*Discussion.* Hemoglobin electrophoresis of the study animal and other bovines suggests (1) the animal was pure yak and not a hybrid, and (2) the yak has a unique Hb among the genus Bos studied (*Hb slow*). Certain sheep greatly increase production of a minor Hb component after prolonged and severe blood loss (13), but no disproportionate Hb production was observed in the yak under various stresses. The two yak Hbs share a common globin chain, and five yaks studied had identical ratios of the two Hbs. Although more observations are required to conclusively rule out Hb polymorphism, these data, plus work on other animals (as reviewed by Hollan *et al.* (14)), suggest that $\alpha$chain duplication is present in the yak. The Hbs are therefore tentatively designated as $^1\alpha_2\beta_2$ (*Hb slow*) and $^2\alpha_2\beta_2$ (*Hb fast*) (Fig. 2). A tetramer of similar globin chains which might have increased oxygen affinity, such as seen in the elephant seal (15) and human Hb H (16) was not found. There may be a difference in the speed of oxidation between *Hbs fast* and *slow*, which, although not proven by the techniques herein, has been suggested by a biphasic oxidation curve found after addition of potassium ferricyanide to yak Hb (17).

The very low rate of reduction of BCB by the yak has been noted before (17). Sheep red cells are deficient in G-6-PD activity, but glutathione reduction is active and there is no hemolysis when challenged *in vivo* with primaquine (18). However, both NADP and NADPH diaphorases influence the BCB test and both have been found to have low activity in cattle (19, 20), whereas specific G-6-PD activity is close to human values (20). Hemolysis and Heinz body formation following ingestion of certain plants or drugs have been reported in cattle (21). It is possible that this also pertains to the yak, as it is of the same genus, but *in vitro* incubation with redox dyes did not produce inclusions in yak erythrocytes.

The observed lack of dependence on 2,3-DPG in altering oxygen affinity has been found in numerous high and low altitude animals, and oxygen affinity has reportedly not been consistently increased or decreased in regard to altitude (23). It has been postulated that oxygen affinity is more closely correlated with body mass (17, 24, 25). A previous report has shown the yak has a $P_{50}$ of 26 mmHg which is similar to that of humans and cows, and an unusual steepness to the upper part of the yak's oxygen dissociaton curve has been suggested as aiding in survival at high altitude (17).

The prompt stabilization of Hb levels after acute blood loss is in contrast to man, where a similar loss without artificial volume replacement might require more than 72 hr for equilibrium to occur (26). Splenic erythrocyte pooling may exist, for the study yak's total blood volume (based on 8% body wt) should have been only 4000 cc, and yet the Hb drop was less than 50% even though 2500 cc of blood was removed. Present data suggest, therefore, that physiologic rather than hematologic adjustments are the primary protective mechanism against acute blood loss in the yak, although cardiac output, tidal volume, DPNH-oxidase systems (27), tissue respiratory pigments (28) and the possible presence of other organic phosphates that might alter oxygen affinity warrant further study. The small size of yak erythrocytes might affect blood viscosity or increased surface area for gas diffusion to the advantage of the yak, but similar indices are found in the Jersey cow (22), and therefore it seems unlikely that this represents a specific form of altitude adaptation.

An observation suggesting that serial influenza A virus infections induced recrudescences of hemolytic anemia due to babesiosis in the yak is presented elsewhere (29).

*Summary.* The yak has two hemoglobins sharing a common globin chain. *Hb slow* appears unique to the yak among the ruminants studied. It is not known if *Hb slow* is important in high altitude adaptation, but physiologic rather than hematologic adjustments may be more important after acute blood loss, as 2,3-DPG remains nil and no disproportionate hemoglobin production occurs.

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