Hemoglobin Types in Barbary Sheep (*Ammotragus Lervia* Pallas, 1777); Absence of a β^C Production in a Homozygous $\beta^{C(na)}$ Animal During Severe Anemia[1] (36559)

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A different hemoglobin, termed hemoglobin C (Hb-C), is produced in sheep, goat, Moufflon, and Barbary sheep during severe blood-loss anemia (1–5). The β chain of this Hb-C differs from the β chains of hemoglobins of nonanemic Caprini in some 10 to 20 positions; the main features of the primary structure of the β^C chain are that the polypeptide is only 141 amino acid residues long, contains one isoleucyl residue and no methionine (6–9). The structures of the β^C chains of anemic sheep, goat, Moufflon, and Barbary sheep are remarkably alike, and only an occasional position is occupied by a different amino acid residue (5, 10). The appearance of Hb-C in anemic sheep occurs only when the animal carries the Hb_{β^A} structural gene; thus, the β chain of Hb-C will replace the β chain of Hb-A but not that of Hb-B which is the product of the allelic Hb_{β^B} locus (4, 6, 11). Such a limitation has not been observed in the other Caprini, because the three β chains of the goat, β^A, β^D and β^E, and the β^B chains of Moufflon and Barbary sheep are known to be replaced by β^C during anemia (3, 5, 12, 13). Most remarkably, however, a second type of hemoglobin of the, nonanemic, adult Barbary sheep contains a β chain which resembles closely the β^C chain of the anemic animal (5, 10). This β chain, termed $\beta^{C(na)}$,[2] differs from the β^C chain by eight amino acid residues only and other characteristic properties of the two chains appear to be the same. We recently had the opportunity to study a Barbary sheep, homozygous for the $Hb_{\beta^{C(na)}}$ structural gene, and found that the production of the $\beta^{C(na)}$ chain cannot be replaced by that of the β^C chain during experimental anemia.

Animals. Blood samples from 18 adult Barbary sheep were kindly made available by Mr. Ralph S. Yohe, Racine, WI; the samples were mailed by air to Augusta, GA. Starch gel electrophoresis showed that 1 Barbary sheep had Hb-B, 7 had Hb-$C^{(na)}$ only, and 10 had both hemoglobins. Two animals, one adult female (No. 504) with Hb-$C^{(na)}$ only and one 10-month-old female (No. 505) with Hb-B and Hb-$C^{(na)}$, were subjected to a severe blood-loss anemia. Both were bled for 6 weeks 300–500 ml twice a week from the jugular vein. Blood samples were collected for examination in vacutainers with EDTA before each bleeding. The recovery phase lasted an additional 16 weeks; during this period five blood collections were made at rather regular intervals. The Barbary sheep were kept outside in cages which allowed the experiment to proceed without undue harm to man and animal.

Methods. Hematological analyses included the determination of packed cell volume [PCV (%)], total hemoglobin (Hb g%), red cell counts ($10^6/mm^3$) and reticulocyte counts (%); standard procedures were used (14). Hemoglobin from red blood cell hemolysate was examined by starch gel electrophoresis in Tris–EDTA–boric acid buffer (pH 9.0) following a previously described procedure (15). Chromatographic analyses made use of 0.9 × 50 cm columns of DEAE-Sephadex (A-50, medium; Pharmacia Fine Chemicals) and Tris–HCl buffers (pH 8.0–7.0) as developers (16). The same procedure, but on a larger scale, was used for preparative purposes. Oxygen equilibria determinations were made on blood samples from Barbary sheep 504 on

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[2] na = nonanemic.

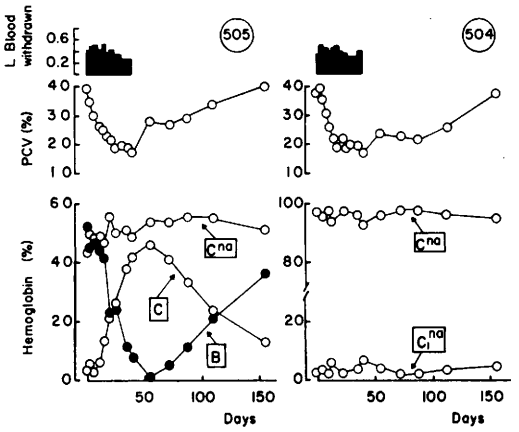

FIG. 1. The relative production of hemoglobins in Barbary sheep during experimental anemia. For details see text.

three occasions, once before the experiment, once 15 days and once 39 days after the start of the phlebotomy. The PCV values of the samples were adjusted to 30% by addition or withdrawal of endogenous plasma. The oxygen dissociation curves were made at 37° at partial CO_2 pressures between 0 and 72 mm Hg using the procedure of Astrup *et al.* (17, 18) and Siggaard-Andersen, Jørgensen and Naeraa (19). Data are presented as the P_{50} values at pH 7.40 and at 37°, *i.e.*, the P_{O_2} required for 50% oxygenation. The level of 2,3-diphosphoglycerate (2,3-DPG) was determined twice a week during the first 4 weeks of the experiment; the method used was that of Grisolia *et al.* (20) and the results are presented in μmoles/g of Hb.

Results. The experimental bleeding produced a severe anemia and the PCV values of the two animals decreased from about 40 to 18% (Fig. 1). The total hemoglobin levels decreased from initial values of 15.3–15.8 g% to 5.9–6.4 g% and the RBC counts dropped from 22.5–23.5 $\times$ 10^6/mm^3 to 5.5–7.5 $\times$ 10^6/mm^3. A mild reticulocytosis was noted between days 10 and 35 and the percentages of reticulocytes varied between 1.5 and 5. The recovery was rapid and uneventful.

Electrophoretic and chromatographic analyses showed that Hb-C$^{(na)}$ in contrast to Hb-B is not replaced by Hb-C during severe anemia. Figure 2 presents chromatograms of hemoglobin of red cell hemolysates obtained before and 35 days after the start of the bleeding experiment. The hemoglobin present in animal 504, who is homozygous for Hb-C$^{(na)}$, is not altered significantly but Hb-B and not Hb-C$^{(na)}$ in animal 505, who is heterozygous for these two hemoglobin types, is almost completely replaced by the anemic Hb-C. The separation of the three variants on DEAE-Sephadex columns was virtually complete thus allowing calculation of the relative quantities. These data are included in Fig. 1 and confirm previous observations that Hb-C will replace Hb-B completely in this animal species during severe blood-loss anemia. The identity of the $\beta^{C(na)}$ chain of the hemoglobin of Barbary sheep 504 before and during anemia was evaluated through analyses of the amino acid compositions of 24 and 72 hr acid hydrolysates. These analyses are of interest because the β^C chain has three more residues of threonine, two more residues of glutamic acid, one more residue each of glycine and valine, two less residues each of aspartic acid and serine and one less residue each of alanine, methionine and lysine than the $\beta^{C(na)}$ chain. Results of these analyses are not presented in detail here; however, the compositions of the β chains of the hemoglobins of animal 504 at day 0 and 35 were almost identical, and not different from the composition of the $\beta^{C(na)}$ chain published earlier (10).

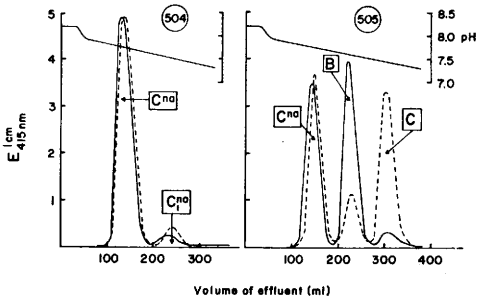

FIG. 2. Separation of hemoglobins from Barbary sheep on columns (0.9 $\times$ 50 cm) of DEAE-Sephadex. Animal 504 is homozygous for Hb-C$^{(na)}$ and animal 505 is heterozygous for Hb-C$^{(na)}$ and Hb-B. (—) Hemoglobins from samples collected prior to the bleeding experiment and (- - -) those from samples collected 35 days after the start of the experiment. C refers to Hb-C produced during anemia.

The oxygen affinity curves of blood samples of Barbary sheep 504 collected before and during the anemia were identical. The P_{50} values at pH 7.4 and 37° varied between 25.6 and 28.1 mm Hg with n values of Hill's equation between 2.85 and 3.0. The levels of 2,3-DPG were low at the start of the experiment, namely 0.3–0.4 μmole/g Hb, and increased slightly to 0.9–1.4 μmole/g Hb. These levels are too low to be of functional significance.

Discussion. Hemoglobin polymorphism in adult nonanemic Barbary sheep has been studied in a limited number of animals. We have been able to obtain data on 34 animals, 18 from a herd in Wisconsin, and 16 from three zoological gardens in Europe. The results show that 7 (21%) were homozygous for Hb-B, 8 (24%) homozygous for Hb-$C^{(na)}$, and the remaining 19 heterozygous for Hb-B and Hb-$C^{(na)}$. Although these numbers are small, they suggest that the genes responsible for the synthesis of the β^B and $\beta^{C(na)}$ chains are allelic.

The data reported in this communication indicate that the β chain of Hb-B but *not* the β chain of Hb-$C^{(na)}$ is replaced by a different β^C chain during severe blood-loss anemia. This situation is somewhat comparable to that in the domestic sheep; the β chain of Hb-A in this Caprini species is replaced by a β^C chain in the anemic animal whereas the β chain of Hb-B is not (4, 6, 11).

Table I compares the minimal numbers of structural differences between the primary structures of the β chain of the three hemoglobin types in the Barbary sheep; the differences between the β^A and β^B chain of the domestic sheep are also listed. Different amino acid residues are found in as many as 21 positions of the β^B and β^C chains (including the four missing amino terminal residues). Similarly, 20 differences have been observed between the β^B and $\beta^{C(na)}$ chains. As many as eight positions in the β^C and $\beta^{C(na)}$ chains are occupied by different amino acid residues; four of these residues in the $\beta^{C(na)}$ chain, namely in positions 50, 55, 56, and 76, are the same as those in the corresponding positions of the β^B chain. No correlation appears to exist between the differences in the struc-

TABLE I. Minimal Numbers of Differences Between Assumed Amino Acid Sequences of Hemoglobin β Chains of Barbary Sheep.[a]

β Chain[b]	2[c]	3	4	5	6	7	10	11	26	50	55	56	58	75	76	84	87	118	120	123	125	129	135	139	144
B	met	leu	thr	ala	glx	glx	ala	val	asx	ser	met	asx	pro	met	lys	thr	glx	his	ser	thr	val	asx	thr	asx	
C	0	0	0	0	pro	asx	leu	ile	glx	thr	leu	gly	ala	val	glx	thr	glx	phe	lys	thr	glx	glx	ala	ser	
C^{na}	0	0	0	0	pro	asx	leu	ile	glx	ser	met	asx	ala	leu	lys	ala	ser	phe	lys	asx	glx	glx	ala	ser	
A/B Sheep[d]										ser/asx			ala/pro	val/met	glx/lys				ser/asx			glx/asx			arg/lys

[a] No distinction is made between aspartic acid and glutamic acid and their amides; these residues are indicated as asx and glx, respectively. Data are from Ref. (5, 10).

[b] Refers to the β chain of Hb-B of nonanemic Barbary sheep; the β chain of Hb-C of anemic Barbary sheep; the β chain of Hb-C of certain nonanemic Barbary sheep.

[c] Residues are numbered following the system used previously (10).

[d] Differences between the β chains of adult sheep hemoglobins A and B.

tures of the β^B and $\beta^{C(na)}$ chain of the Barbary sheep and those of the β^A and β^B chains of the domestic sheep.

In reviewing these data the following paradox becomes apparent: (a) The similarity of the primary structures of the $\beta^{C(na)}$ and β^C chains suggests that the Hb_{β^C} and $Hb_{\beta^{C(na)}}$ structural genes are alleles; the absence of a β^C production in an animal homozygous for the $Hb_{\beta^{C(na)}}$ gene is concordant with that assumption. (b) The survey data on adult Barbary sheep indicate allelism between the Hb_{β^B} and the $Hb_{\beta^{C(na)}}$ structural genes; the β^B chain, however, is replaced by the β^C in the anemic BB or $BC^{(na)}$ animal.

One possible explanation for this complex mechanism may be found in the assumption that (a) the Hb_{β^C} and $Hb_{\beta^{C(na)}}$ structural genes are alleles; (b) the Hb_{β^B} locus arose from the Hb_{β^C} locus, or vice versa, through the process of duplication and a subsequent series of mutations; (c) the activity of the Hb_{β^C} locus is decreased greatly but can be stimulated by unknown means during the stress of anemia; (d) the activity of the $Hb_{\beta^{C(na)}}$ locus in a heterozygous animal is similar to that of a functioning Hb_{β^B} structural gene because a comparable Hb_{β^B} locus on the chromosome with the $Hb_{\beta^{C(na)}}$ structural gene is completely suppressed. Thus, in a heterozygous $BC^{(na)}$ Barbary sheep a $Hb_{\beta^B} + Hb_{\beta^{C(silent)}}$ cistron would be allelic with a $Hb_{\beta^{B(silent)}} + Hb_{\beta^{C(na)}}$ cistron.

It is also possible that the presence of the Hb_{β^C} gene on a chromosome with the Hb_{β^B} locus occurred because of a crossover between a chromosome carrying the Hb_{β^B} locus and another chromosome with a specific $Hb_{\beta^{C(na)}}$ locus. Subsequent mutations of this $Hb_{\beta^{C(na)}}$ locus caused changes which are presently recognized by the structural differences between the β^C and $\beta^{C(na)}$ chains as well as by differences in production rates of the two genes. In an arrangement like this the $Hb_{\beta^B} + Hb_{\beta^C}$ cistron and the $Hb_{\beta^{C(na)}}$ cistron would act as allelic genes.

Summary. Hemoglobin polymorphism in the Barbary sheep is reviewed. Electrophoretic, chromatographic, and limited structural analyses have demonstrated that during blood-loss anemia Hb-B of this animal species is replaced by another type, termed Hb-C, whereas a Hb-C like variant, termed $Hb\text{-}C^{(na)}$ and present in certain nonanemic Barbary sheep, cannot be replaced when the animal is made severely anemic. Possible genetic mechanisms responsible for these phenomena are discussed.

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