Higher oxygen affinity of sheep Hb C compared to Hb A and Hb B[1]

A. M. Vaccaro-Torracca, R. Vestri and S. Salmaso

Laboratorio di Patologia non infettiva, Istituto Superiore di Sanità, Viale Regina Elena 299, Rome (Italy), and Istituto di Fisiologia Umana, Università Cattolica S. Cuore, Via della Pineta Sacchetti 644, I-00168 Rome (Italy), 1 August 1979

Summary. Sheep which have Hb A, synthesize the perinatal Hb C in response to severe tissue hypoxia. It is known that Hb A displays a higher oxygen affinity than Hb B. The results of this study indicate that Hb C exhibits an oxygen affinity and a Bohr effect higher than those of Hb A and Hb B.

Adult sheep possess either Hb A or Hb B or both. The beta chains of Hb A and Hb B differ in 7 amino acid residues[2]. Sheep possessing Hb A, under severe erythropoietic stress, synthesize Hb C (produced for a short time after birth[3]) while simultaneously repressing the synthesis of Hb A[4]. Hb C has a 3rd type of beta chain which differs from beta^A and beta^B chains by 16 and 21 amino acid residues respectively[2]. It has been known for a long time that Hb A displays a higher oxygen affinity than Hb B, while the oxygen affinity of Hb C does not differ significantly from that of Hb A[4]. The present study reinvestigates the respiratory properties of Hb C as well as those of Hb A and Hb B by a method which allows simultaneous determinations of oxygen affinity on different samples.

Materials and methods. Hb C was obtained from a sheep of the Ile-de-France breed, homozygous for Hb A (No. 585, weighing 72 kg), during anemia induced by phenylhydrazine. Hb A was obtained from the 585 sheep and from a sheep of the Sopravvissana breed, heterozygous for Hb A and Hb B (No. 771, weighing 40 kg), which also provided Hb B.

The hemolysates (200–250 mg of Hb) were resolved into the individual hemoglobins by chromatography on a DEAE Sephadex A-50 column, 1.5 × 60 cm, using a 500 ml + 500 ml linear gradient of decreasing pH (8.2–7.5 range) in 0.05 M Tris-HCl buffer. The purity of the 3 hemoglobin preparations was checked by electrophoresis on cellulose acetate plates at pH 8.4.

To measure the oxygen affinity, the hemoglobin solutions (1.5×10^{-5} M), contained in 3 ml automatic flux cuvettes, were equilibrated with known O_2–N_2 mixtures by bubbling for 5 min at constant temperature. The oxygen saturation was determined by a 2-wavelength technique[5]. The wavelengths 576 and 560 nm were chosen because, at these values, a good sensitivity and a low interference from methemoglobin are obtained. Samples containing more than 2% of methemoglobin, as tested by the method of Benesch[6], were discarded. Each oxygen affinity curve was constructed with at least 4 points. Spectrophotometric measurements were made with a Beckman Acta III Spectrophotometer equipped with 10-mm light path cuvettes kept at steady temperature. The buffer was 0.05 M Bis-Tris in 0.1 M NaCl and the pH values were measured at the temperature of each experiment.

Results and discussion. The relationship between log P_{50} of Hb A, Hb B and Hb C and pH at 37 °C, is shown in figure 1. Hb C exhibits a higher oxygen affinity than Hb A and Hb B at all pH examined. The values for the Bohr effect in the range between pH 6.89 and pH 7.37 are: −0.32 for Hb A, −0.36 for Hb B and −0.56 for Hb C.

The temperature dependence of the binding of hemoglobins with oxygen was also investigated. In figure 2 the log P_{50} measured at 15, 20, 27, 34 and 37 °C at a constant pH of 7.25 is shown. The heats of reaction (ΔH) of hemoglobins A, C and B are: −7.3, −7.1 and −6.2 respectively, indicating that a rise in temperature (muscular exercise) favours a dissociation of Hb A and Hb C more than that of Hb B, as found by other authors[7].

P_{50} values of the 3 hemoglobins obtained at 37 and 20 °C (pH 7.25) are shown in the table and are compared with

P_{50} of sheep hemoglobins A, B, C at different temperatures

	37 °C pH 7.25	20 °C pH 7.25	20 °C pH 7.20*
Hb B	31.1	17.0	16.0
Hb A	24.6	12.4	10.1
Hb C	17.2	8.9	8.8

* H.F. Bunn et al.[8].

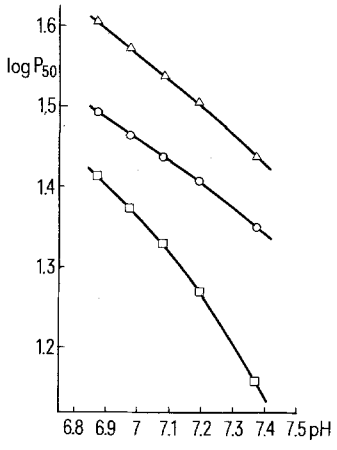

Fig. 1. Effect of pH on log P_{50} of Hb A (○); Hb B (△); Hb C (□); T = 37 °C. The buffer is 0.05 M Bis-Tris in 0.1 M NaCl. Hb concentration is 1.5×10^{-5} M.

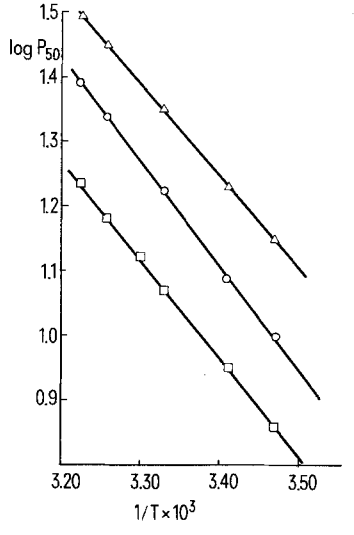

Fig. 2. Log P_{50} as a function of 1/T. Hb A (○); Hb B (△); Hb C (□). Buffer and Hb concentration as in figure 1.

those reported by Bunn[8] at 20 °C. The values at this temperature are very similar, although a more pronounced difference between the oxygen affinity of Hb A and Hb C is observed. This difference is more markedly increased at 37 °C. The P_{50} detected at 37 °C for Hb A and Hb B are, on the other hand, in good agreement with those derived from data obtained by Bauer[7] (who did not analyze Hb C). Other investigators, who examined the oxygen affinity of Hb C, as well as that of Hb A and Hb B at 37 °C, did not find a significant difference between Hb C and Hb A in whole blood and in hemolysates[9], although in a previous paper they reported a shift of the affinity curve to the left for whole blood containing Hb C[10]. The discrepancy between these results and ours can be ascribed to the different experimental conditions, for example the method of purification of the hemoglobins, the composition of the buffer, the presence of CO_2, and the method used to determine the oxygen saturation.

A larger Bohr effect of Hb C compared to Hb A and Hb B has already been reported, but determined in presence of CO_2[9] (our experiments were performed in absence of CO_2). The findings that a hemoglobin with increased oxygen affinity is produced when hypoxia is of such a degree to put in danger the life of the animal is apparently intriguing. In fact, man and other mammals (dog, horse, pig) which possess hemoglobins with intrinsically high oxygen affinity[8] adapt to hypoxia by lowering the oxygen affinity through the increase of intracellular 2,3-DPG. On the other hand, it is possible that sheep, which have hemoglobins with intrinsically low oxygen affinity that do not bind 2,3-DPG[8], could benefit from the production of a hemoglobin with a raised oxygen affinity and a large Bohr effect. Even in cats, which also have a hemoglobin with low oxygen affinity, phenylhydrazine-induced anemia results in an increase of hemoglobin components with high oxygen affinity[11]. A better resistance to extreme hypoxia was reported in mice, when the oxygen affinity of the hemoglobin was artificially increased[12].

1 Part of this work was supported by a grant of Ministero Pubblica Istruzione.
2 S.H. Boyer, P. Hathaway, F. Pascasio, C. Orton, J. Bordley and M.A. Naughton, Science *153*, 1539 (1966).
3 T.H.J. Huisman, J.P. Lewis, M.H. Blunt, H.R. Adams, A. Miller, A.M. Dozy and E.M. Boyd, Pediat. Res. *3*, 189 (1969).
4 G. van Vliet and T.H.J. Huisman, Biochem. J. *93*, 401 (1964).
5 E.J. van Kampen and W.G. Zijlstra, Adv. clin. Chem. *8*, 141 (1965).
6 R. Benesch, G. Mac Duff and R.E. Benesch, Analyt. Biochem. *11*, 81 (1965).
7 C. Bauer and H.D. Jung, J. comp. Physiol., B *102*, 167 (1975).
8 H.F. Bunn, U.S. Seal and A.F. Scott, Ann. N.Y. Acad. Sci *241*, 498 (1974).
9 T.H.J. Huisman and J. Kitchens, Am. J. Physiol. *215*, 140 (1968).
10 S.L. Moore, W.C. Godley, G. van Vliet, J.P. Lewis, E. Boyd and T.H.J. Huisman, Blood *28*, 314 (1966).
11 A.G. Mauk, H.T. Whelan, G.R. Putz and F. Taketa, Science *185*, 447 (1974).
12 J.W. Eaton, T.D. Skelton and E. Berger, Science *183*, 743 (1974).

Improved response to heat after moderate physical training in man[1]

J.H.M. Bittel and A.G.C. Buguet[2]

Centre de Recherches du Service de Santé des Armées, Division de Physiologie, 108 Boulevard Pinel, F-69272 Lyon Cedex 1 (France), 18 June 1979

Summary. 7 young men marched for 6 days (35 km daily) in a cool climate at about 35% of their $\dot{V}O_2max$. Sweat output was measured at rest in a climatic chamber during a controlled hyperthermia test performed before and after the trial. 4 subjects served as controls. The main finding of the study was that sweat output increased 17.3% ± 1.5 SEM on the post-trial test, without any change in $\dot{V}O_2max$. It is concluded that moderate physical training can improve heat responses in resting man.

Since Wyndham's report in 1951[3], acclimatization to heat has been described as occurring in man working in hot environments[4,5]. Heat acclimatization has also been achieved both by repeated passive heating sessions in resting man[6,7] and by physical exercise performed in a cool climate, followed by increased maximal aerobic consumption ($\dot{V}O_2max$)[8–13]. Such results were discussed by Hale[14] and Glaser and Shephard[15] as intervening factors in 'cross adaptation'. However, the specific role of physical exercise in the processes of response to heat has not yet been clarified, since the procedures to test the thermoregulatory response to heat in most of these studies have associated work and heat exposure, and the exercises performed always led to increased physical fitness. Our aim was to eliminate this latter factor by using a method involving a moderate repetitive physical exercise in a cool environment which did not result in a change in physical fitness, and by testing the thermolytic responses to heat in the subject at rest.

Methods. a) The subjects, 12 male Caucasian volunteers, aged 20, were separated into 2 groups. Group 1 consisted of 8 subjects who participated in a field trial in cool weather conditions (ambient temperature = 0–7 °C). This trial consisted of a 6-day walk at 5.6 km · h^{-1} on a flat terrain at 300 m altitude, during 7–8 h a day. The subjects wore standard army clothing and carried a backpack suitably weighted to allow an energy expenditure of approximately 35% of each one's maximal oxygen consumption ($\dot{V}O_2max$ ranging from 48.3 to 62.4 ml $O_2 \cdot min^{-1} \cdot kg^{-1}$)[16]. Group 2, which consisted of 4 sedentary control subjects, performed daily routines throughout the experimental period.

b) The thermal test consisted of a controlled hyperthermia test performed in a climatic chamber[6], the subjects lying nude on a string bed allowing sweating and evaporation. In order to reach steady body temperatures they spent their first 90 min in a thermoneutral environment. The ambient temperature was then raised to reach the target internal temperature (tympanic temperature = 38 °C), which was maintained for 1 h by ambient air humidity adjustments. The sweat loss during the hour of steady hyperthermia was measured by weighing the subject continuously.

c) Experimental procedure: in group 1, the thermoregulatory responses to heat were examined 3 weeks before (pre) and immediately after (post) the field trial (making an