Determination of Hüfner's Factor and Inactive Hemoglobins in Human, Canine, and Murine Blood

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Summary Determination of Hüfner's factor and simultaneous measurement of met- and carboxyhemoglobin were performed in 31 human, 16 canine, and 2 murine blood samples. Both measured and corrected values of Hüfner's factor were lower than the theoretical value of 1.39, suggesting the possibility of the presence of unidentifiable inactive hemoglobin(s) in blood.

Key words: Hüfner's factor, inactive hemoglobin, O₂ capacity.

Hüfner's factor is the oxygen binding capacity per 1 g of hemoglobin, which was first reported by Hüfner in 1894 (HÜFNER, 1894). The value was theoretically calculated as 1.34 from the iron-equivalent molecular weight of and stoichiometry of oxygen binding to hemoglobin and was also experimentally determined as 1.338 by combined use of gasometric and spectrophotometric procedures (HÜFNER, 1894). The theoretical value, however, should be 1.39, based on the currently known molecular weight of hemoglobin. After Hüfner's first estimation, a number of variable values have been reported, and most of them are lower (GREGORY and MILLAR, 1973; GREGORY, 1974; DIJKHUIZEN *et al.*, 1977) and a few are higher than the theoretical value (GREGORY *et al.*, 1971). Moreover, the same reporter published very different values at different times (GREGORY *et al.*, 1971; GREGORY and MILLAR, 1973; GREGORY, 1974). Recently, DIJKHUIZEN *et al.* (1977) published an extensive and critical study, but their values, too, were a little lower than 1.39. Here we attempted this study for the clarification of the following problems: 1) Is the actually determined value lower than the theoretical one? 2) If so, what is the reason? 3) Does an unidentified inactive hemoglobin really exist, as DIJKHUIZEN *et al.* (1977) assumed as a cause of the lower value? In addition, this study may offer a simple and accurate means of determining the oxygen capacity of blood from its hemoglobin content.

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Blood was obtained from 31 healthy adults (27 males and 4 females, ranging in age from 19 to 55), 16 mongrel dogs (3 males and 13 females), and 8 mice (20-week-old males). No donor was medicated within 29 h before blood drawing. All measurements were completed within 7 h after the sample was obtained from the cubital vein in humans, from the foreleg vein in dogs, and from the carotid artery in mice. In the two determinations of mice, pooled blood from 2 groups of 4 mice each was used. Approximately 3 ml of the heparinized blood was equilibrated with air for 20 min in a glass tonometer which was gently rotated in a water bath at room temperature. After equilibration, the blood was promptly taken into glass syringes and was kept in ice water until use.

Oxygen content of the blood was determined by a micromanometric method of VAN SLYKE and PLAZIN (1961) using 0.1 ml of sample. Measurements were usually performed in duplicate, or more, on the same samples. Physically dissolved oxygen was read from published data (VAN SLYKE and PLAZIN, 1961) and subtracted to obtain the oxygen capacity. To assure more accurate determination of the gas, the apparatus and the needle-tipped micropipets were used after calibration of the instrumental volume ratio, R_v :

$$R_v = a/b = [\text{Gas}]/(P \times f_t), \quad (1)$$

where a , volume of gas in the chamber of the apparatus when P is measured; b , volume of sample measured from one of the micropipets, $[\text{Gas}]$, mmol of gas per l of samples assayed; P , gas pressure (mmHg), and f_t , a temperature factor. For the actual calibration, the following chemical reaction which evolved N_2 gas was used (SENDROY, 1937):

Hence, we can replace 1.5 $[\text{KIO}_3]$ for $[\text{Gas}]$ in Eq. (1) and calculate R_v as

$$R_v = 1.5[\text{KIO}_3]/(P_{\text{N}_2} \times f_t), \quad (3)$$

where $[\text{KIO}_3]$ is the concentration (mmol) of the standard KIO_3 solution used and P_{N_2} is the observed N_2 pressure (mmHg).

Accurate hemoglobinometry is extremely important for the precise determination of Hüfner's factor, and we used the cyanomethemoglobin method with millimolar extinction coefficient of 11.00 at 540 nm (on heme basis) (ICSH, 1978) for this purpose. In view of recent reviewers' comments (TENTORI and SALVATI, 1981), special care was taken in the exact dilution of blood samples. Carboxyhemoglobin (HbCO) content was measured by the method of COMMINS and LAWTHORP (1965), and methemoglobin (Hb^+) content by a presently developed difference spectrum method in near infrared region (ENOKI *et al.*, unpublished). The method is based on the fact that under acid pH Hb^+ has an absorbance peak around 630 nm which disappears upon addition of CN^- to convert Hb^+ to cyanomet form. With this method one can measure blood Hb^+ of less than 1% with

Table 1. Hüfner's factors and inactive hemoglobins of fresh human, canine, and murine blood.

Blood	Hüfner's factor		Hb ⁺ (%)	HbCO (%)	Hbx (%)
	Measured	Corrected*			
Human (n=31)	1.361 ± 0.027	1.375 ± 0.027	0.34 ± 0.10	0.40 ± 0.42	1.28 ± 1.83
Canine (n=16)	1.336 ± 0.040	1.386 ± 0.037	3.01 ± 2.73	0.29 ± 0.26	0.58 ± 2.23
Murine (n=2)	1.359	1.364	0.33	0.06	1.84

Results are mean ± S.D. except for murine blood. * Corrected for the presence of Hb⁺ and HbCO in blood.

Fig. 1. Frequency distributions of met- and carboxyhemoglobin contents in human and canine blood samples. Ordinates: frequency (*n*), abscissae: HbCO% (left) and Hb+% (right).

a sufficiently high accuracy and reliability.

As shown in Table 1, the measured values of Hüfner's factor were apparently lower than the theoretical one (1.39). The average value for humans was very close to those reported by DIJKHUIZEN *et al.* (1977), i.e., 1.368. In dogs the value was much less than the theoretical, with rather large variations. It can be assumed that the lower values are caused primarily by a partial conversion of hemoglobin to such derivatives as Hb⁺ and HbCO incapable of reversible oxygen binding. In both humans and dogs, blood HbCO levels were relatively low with small variations (Table 1, Fig. 1), and the value in humans was within normal range (0.2 to 1.5%) as reported by previous studies (RODKEY *et al.*, 1979). Distinctly higher values were

found only in two heavy smokers (Fig. 1). Although average Hb^+ level in the present human blood samples was a little lower than, but not far from, the normal range (0.5 to 1.0%) in a previous report (RODKEY and O'NEAL, 1974), the levels in canine blood were considerably higher and variable. This result suggests that the low and variable values in the Hüfner's factor of canine blood might principally reflect the high and variable contents of Hb^+ . The Hüfner's factor was still a little lower than the theoretical one even after being corrected for the presence of HbCO and Hb^+ (Table 1). This implies and confirms the possibility that an unidentified hemoglobin(s), Hbx , may be present in blood (DIJKHUIZEN *et al.*, 1977). The nature of Hbx is obscure. Possibilities of sulfhemoglobin and incomplete oxygenation of blood for the lower Hüfner's factor can be neglected by the following results of DIJKHUIZEN *et al.* (1977): 1) The low value was observed even after tonometry of blood with 100% oxygen for 150 min, and actually no significant difference was found between the values obtained after tonometry with $P_{\text{O}_2} \approx 650$ mmHg and $P_{\text{O}_2} \approx 150$ mmHg (1.351 ± 0.023 vs. 1.359 ± 0.020 , $n=5$), and 2) the sulfhemoglobin content was negligible in most of normal blood samples.

Practically, the Hüfner's factor for any blood samples can be assessed if the total Hb and Hb^+ content are determined. Since the HbCO content varies little from sample to sample (Fig. 1) and one can assume a constant value of it, say 0.40% (Table 1), we can conveniently determine the oxygen capacity of blood from actually measured hemoglobin content of blood and the assumed value of Hüfner's factor.

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