Upsala J Med Sci 83: 7–16, 1978

Total Body Haemoglobin Estimated with the Alveolar CO Method as Compared with a ^{51}Cr Technique

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ABSTRACT

Total body haemoglobin was estimated by the alveolar equilibration CO method and by dilution of ^{51}Cr-tagged erythrocytes in 22 patients with a wide range of haemoglobin concentrations (51–190 g/l). The resulting regression equation: $THB_{CO}=47+0.81\times THb_{Cr}$, where THn is expressed in grams, shows that with increasing THb successively lower values were obtained with the THb_{CO} method as compared with the THb_{Cr} method. Individual values were calculated for the M-factor, i.e. the ratio of the haemoglobin affinities to O_2 and CO. These values were positively and significantly correlated to the red-cell content of 2.3-diphosphoglycerate. The findings are consistent with a recent hypothesis that the effect of 2.3-DPG on CO affinity may not be equivalent to its effect on oxygen affinity. The discrepancy between the two methods of estimating THb may therefore be apparent only and due to a systematic variation in the M-factor.

INTRODUCTION

Carbon monoxide (CO) was first used as a label for blood volume estimation nearly 100 years ago (22). However, the method was not put into clinical practice until 1948, when Sjöstrand described his alveolar equilibration CO technique for the determination of the total haemoglobin content of the body (38). Since then several modifications have been published. All have in common with the Sjöstrand method the injection of a comparatively small and known amount of CO and measurement of the carboxyhaemoglobin (COHb) concentration before as well as after the injection. The original Sjöstrand method has a certain appeal to the clinician, being a bloodless technique utilizing only one method for CO determination. The published comparisons between this method and methods utilizing dilution of dyes or labelled erythrocytes have shown good agreement (14, 19, 29, 31, 34, 45). However, with one single exception (45), all studies in humans have been confined to individuals with haemoglobin (Hb) concentrations within the normal range. The importance of extending such studies to include also anaemic and polycythaemic cases has been actualized by some recent findings. The estimation of total haemoglobin (THb) using the alveolar CO method requires that the affinity of Hb for CO is known or constant between individuals. In the presence of increased concentrations of 2.3-diphosphoglycerate (2.3-DPG), adult Hb shows a decrease in the oxygen affinity (3, 10). This is the case for example in subjects with chronic anaemia, heart disease and hypoxic hypoxaemia (12, 23, 43). Our knowledge of the influence of 2.3-DPG on CO affinity is at present less advanced (8). Some observations on foetal haemoglobin indicate that the effect of 2.3-DPG on CO affinity may not be equivalent to its effect on oxygen affinity (15). If so, it cannot be excluded that the ratio of the affinities to oxygen (O_2) and carbon monoxide might vary with the erythrocyte concentration of 2.3-DPG and therefore with the haemoglobin concentration. Due to the paucity of data covering the reliability of THb estimations using the alveolar CO method (THb_{CO}) in subjects with abnormal Hb levels the problem was reinvestigated. Estimations of THb were performed on patients having haemoglobin values over a wide range using a technique with dilution of ^{51}Cr-tagged erythrocytes in parallel with the alveolar CO technique. In most patients, determinations of the intra-erythrocyte 2.3-DPG content were also performed.

MATERIAL AND METHODS

The entire material consists of 33 patients. The age and sex distributions and the diagnoses are seen in Table I a and b. For reasons mentioned below the THb_{CO} estimations were discarded as unreliable in 11 cases (Table I b).

Table I

Case	Diagnosis	Sex (f/m)	Age (years)	Weight (kg)	Hb (g/l)	Hct (%)	2.3 DPG moles×10^3/l of erythrocytes	Initial COHb (%)
Table I a								
1	Iron def. anaemia	f	52	66.4	99.2	30.5	7.30	0.46
2	Iron def. anaemia	f	49	68.1	95.5	29.5	6.85	0.67
3	Iron def. anaemia	f	49	75.9	88.5	29.0	6.61	0.61
4	Iron def. anaemia	f	54	64.2	73.0	26.0	6.91	0.74
5	Iron def. anaemia	f	37	59.2	96.2	32.7	–	0.51
8	Iron def. anaemia	f	48	65.0	79.9	27.0	6.70	0.63
11	Multiple myeloma	m	58	61.4	50.9	15.5	–	0.55
12	Chron. lymph. leukemia	m	66	70.5	57.6	17.0	10.10	0.82
13	Macrocytic anaemia	f	84	49.5	60.5	22.0	7.40	0.51
14	Polycythaemia vera	f	75	49.5	181.3	52.0	–	0.65
15	Pericarditis	m	67	85.2	171.1	57.3	–	0.86
17	Thromboang. oblit.	m	50	65.2	142.0	43.0	–	0.55
18	Arterial hypertension	m	31	72.0	132.4	40.0	–	0.58
19	Eisenmenger's complex	f	38	47.0	165.8	62.0	6.94	0.79
20	Polycythaemia vera	f	45	60.8	94.4	30.0	–	0.67
21	Normal	m	62	91.4	179.7	55.3	5.05	0.99
23	Arterial hypertension	m	61	75.2	179.7	52.5	6.24	0.82
25	Normal	m	27	99.2	161.6	45.8	5.13	0.73
30	Arterial hypertension	f	72	70.2	147.0	46.3	5.94	0.66
31	Polycythaemia vera	m	54	64.1	167.2	50.5	5.05	0.79
32	Tetralogy of Fallot[a]	m	48	58.0	190.4	59.0	3.98	0.63
33	Ischemic heart disease	m	39	89.0	163.0	51.0	5.04	0.63
Table I b								
6	Myxedema	f	40	62.7	105.7	32.2	4.84	1.15
7	Chron. lymph. leukemia	m	64	63.5	49.8	19.3	–	2.12
9	Erythrocytosis	m	22	70.2	207.8	68.0	5.15	2.84
10	Polycythaemia vera	m	65	52.5	73.9	19.5	–	1.28
16	Diabetes mellitus	m	74	78.0	174.9	49.5	–	2.31
22	Polycythaemia vera	m	74	75.5	169.3	53.5	6.48	1.13
24	Diabetes mellitus	m	21	63.0	167.5	48.3	–	3.72
27	Polycythaemia vera	f	62	56.2	186.0	56.7	4.49	1.12
28	Haemolytic anaemia	m	26	66.2	59.0	19.0	8.44	2.69
29	Erythrocytosis	m	66	92.2	188.4	55.0	5.39	1.10
26	Myeloid metaplasia	f	76	51.0	67.4	19.3	–	–

In the remaining group of 22 patients (Table I a) no respiratory disorder was known or suspected and all patients were in a condition that enabled them to cooperate adequately during the rebreathing procedure. A slight to moderate enlargement of the spleen was found in the leukaemic and some of the polycythaemic patients and also in the patient with haemolytic anaemia. A significant splenomegaly was found only in the patient with myeloid metaplasia.

The THb_{CO} was estimated on 2 successive days and the THb_{Cr} in close succession to one of these measurements. In one patient (No. 26) the rebreathing procedure had to be interrupted for technical reasons. The THb_{CO} estimations were performed according to Sjöstrand (38) with some modifications (25). The rebreathing apparatus AGA ME-1550 (AGA, Stockholm) was used. In accordance with the procedure used by Strandell (41) only one rebreathing bag was collected after the CO administration. The capacity of the bag was 6.5 litres. When measuring the actual gas volume in 8 different bags after rebreathing 15 minutes and after maximal expiration the mean volume in 10 individuals was 5.5 litres (range 4.6–6.0). Before each THb_{CO} determination the apparatus was checked for leakage, using a water manometer. The oxygen consumed during the periods of equilibration was replaced by refilling the bag wigh oxygen every 7½ min.

The oxygen fraction of the gas in the bag was analysed in a Beckman Oxygen Analyser Model C-2. Each day before use, the accuracy of the oxygen analyser was checked with two gases, viz. 85% O_2 in N_2 and 100% O_2, with correction for the actual barometric pressure. If the oxygen fraction in a bag after rebreathing was lower than 0.90, the test was considered unreliable due to leakage and was discarded. This happened in 2 cases (Nos. 27 and 29). The CO was analysed on an infrared (IR) instrument (URAS 2, Hartmann & Braun AG, Frankfurt/Main), using the wavelength 4 500 nm. The bags from all but 8 patients were also analysed on a CO-meter (type SL 2, Stålex, Stockholm) with a modification of the hopcalite method developed by Sjöstrand & Lindelöw (37). There was no

THb CO-IR (g)	THb CO-Stålex (g)	THb Cr (g)	RCV Cr (ml)	M calc.
359	370	400	1 228	206.5
368	386	418	1 291	202
351	345	360	1 177	225
223	221	218	766	235
312	311	345	1 172	208
335	352	325	1 077	237
198	198	238	757	190.5
372	360	316	929	275
135	140	133	486	235
503	534	593	1 714	194
783	726	808	2 759	222.5
602	617	694	2 083	199
543	578	628	1 854	201.5
544	534	642	2 359	194
290	289	316	1 007	233
854	856	1 009	3 099	193.5
712	704	799	2 398	203
763	773	943	2 646	210
478	–	478	1 593	231
642	–	823	2 421	178.5
638	–	745	2 305	196.5
817	–	892	2 581	211
382	366	402	1 195	218
332	335	262	993	298
1 114	1 020	1 354	4 440	183.5
196	189	303	972	146
758	719	958	2 667	179
747	717	739	2 243	233
516	467	578	1 671	212
505	–	740	2 188	155
331	–	261	908	295.5
776	–	889	2 615	200.5
–	–	77	666	–

significant difference between the THb values when the gas was analysed with the IR and the hopcalite techniques ($\bar{d}$=+2.6±3.8 g, n=33). The precision of the THb determinations was approximately the same for the IR technique (3.3%) and the hopcalite technique (3.5%) for gas analyses. For all calculations presented in Tables and Figures the values obtained with the IR technique were used.

Two reference gases with CO concentrations of about 50 and 100 ppm were used. In order to ascertain a high accuracy of the CO analysis one of the reference gases was analysed by the manufacturer (AGA, Stockholm) as well as by another laboratory (courtesy of Dept. of Clinical Physiology, The Thoracic Clinics, Karolinska Sjukhuset, Stockholm). These values were not known until another series of analyses had been performed at our laboratory. At AGA, the gas was analysed with an IR technique and an iodine pentoxide technique (20). At the two Departments of Clinical Physiology, the reference gas was checked against ten different gas mixtures prepared at each laboratory. In each procedure the reference gas and one gas mixture were analysed 10 times with the hopcalite technique (Stockholm) or an IR technique (Uppsala). Thus the total number of analyses of the reference gas was more than 200. The following results were obtained. AGA, Stockholm: 47.0 ppm (range 46.5–47.4). The Thoracic Clinics, Stockholm: 46.4 ppm (coefficient of variation 2.2%). Our laboratory: 46.5 ppm (coefficient of variation 2.1%).

The precision of our analyses with the IR technique was 0.9% and with the hopcalite technique 1.8%. The linearity of both the IR and the hopcalite method was tested repeatedly in the range 20–200 ppm CO in air. All these tests have shown an excellent linearity of these two methods (correlation coefficients 0.9992–0.9996). In addition the linearity of the instrument in each THb_{CO} estimation was calculated from readings using the reference gases according to the formula:

$$\frac{\text{Gas conc.}_{\text{high}}}{\text{Gas conc.}_{\text{low}}} = \frac{\text{reading}_{\text{high conc.}}}{\text{reading}_{\text{low conc.}}} \times K$$

The factor K was within the range 0.9–1.1 in all but two cases (Nos. 12 and 20) of the selected material. As is seen from Table I*a*, these two cases contributed less than the average to the discrepancy between the two methods and the THb_{CO} values obtained with the infrared and the hopcalite techniques were almost identical. The THb_{CO} was calculated according to the Formula 1.

M =231=empirical constant
$F_{O_{2\,I}}$ =Fraction of O_2 in rebreathing system prior to administration of CO
$F_{O_{2\,II}}$ =Fraction of O_2 in rebreathing system after administration of CO
F_{CO_I} =Fraction of CO in rebreathing system prior to administration of CO
$F_{CO_{II}}$ =Fraction of CO in rebreathing system after administration of CO
P_B =Barometric pressure, kPa
K_{Mb} =0.95=correction factor for extravascular CO
f_{STPD} =Factor for converting gas volumes to STPD
V_{CO_i} =Volume of CO administered to rebreathing system ATP
F_{CO} =Fraction of CO in administered gas, at present approx. 0.995

Formula 1.

$$THb_{CO} = \frac{f_{STPD} \times 100 \times K_{Mb} \left[V_{CO_i} \times F_{CO_i} - F_{CO_{II}} (V_{app} + V_{RV} + V_S) - F_{CO_I} (V_{app} + V_{RV}) \right]}{1.39 \left[\frac{M \times 100 \times F_{CO_{II}} (P_B - 6.3)}{F_{O_{2\,II}} (P_B - 11.6) + M \times F_{CO_{II}} (P_B - 6.3)} - \frac{M \times 100 \times F_{CO_I} (P_B - 6.3)}{F_{O_{2\,I}} (P_B - 11.6) + M \times F_{CO_I} (P_B - 6.3)} \right]}$$

V_{app} = Volume of rebreathing apparatus approx. 3 litres ATP
V_{RV} = Residual volume of patient, approx. 1.5 litres ATP
V_S = Volume in rebreathing bag after max. expiration, approx. 5.5 litres ATP
1.39 = Oxygen binding capacity of haemoglobin

In order to eliminate the influence of environmental CO sources, tobacco smoking etc., all THb_{CO} estimations with an initial COHb concentration of more than 1.0% were discarded. This happened in 8 cases (Nos. 6, 7, 9, 10, 16, 22, 24 and 28).

The determinations of THb and circulating red cell volume (RCV) with the ^{51}Cr technique were performed using autologous red blood cells according to a method described in detail previously (6). Injection of the labelled cells was made in an antecubital vein. After injection, 3–8 venous blood samples were withdrawn, avoiding stasis, from the contralateral arm. In cases of polycythaemia and splenomegaly or heart disease the last sample was taken about 1 hour after injection. Mixing of the labelled red cells was assumed to be complete when their concentration in at least three successive samples was constant (±3%). The accuracy and precision of the method was checked repeatedly during this study by model experiments *in vitro* and the results were in accordance with those previously obtained (6). The amount of ^{51}Cr injected into each patient was 0.1 μCi/kg body weight. This will give an absorbed dose of 5–10 mrads to the blood and 0.8 mrads to the whole body. $Na_2{}^{51}CrO_4$ was obtained from Atomenergi AB, Studsvik, Sweden.

The determinations of haemoglobin concentration were performed in triplicate spectrophotometrically after conversion of haemoglobin to cyanmethaemoglobin with "Aculute" (Ortho Pharmaceutical Corp., New Jersey, USA). The accuracy of the method was checked by regular analyses of haemoglobin cyanide standard solutions (E. Merck, Darmstadt, West Germany). According to current recommendation (13, 27, 40) 64458 was used as the molecular weight of Hb and 11.0 as the millimolar extinction coefficient of cyanmethaemoglobin at 540 nm.

The haematocrit readings were performed in triplicate using an International Microcapillary centrifuge, Model MB. The centrifugation time was 5 minutes. Using this centrifuge the amount of trapped plasma is 1.7% after centrifugation for 3 min and 1.3% after 10 min. No correction for trapped plasma was made. 2.3-DPG was determined by Bartlett's chromatographic method (2) as modified by deVerdier & Killander (11). The determinations were performed at the Department of Clinical Chemistry, University Hospital, Uppsala, by courtesy of Dr Magnus Hjelm.

RESULTS

Table I *a* and *b* shows for all patients the individual values of haemoglobin concentration, haematocrit, erythrocyte content of 2.3-DPG, the initial values of COHb concentration, and the THb_{CO} and THb_{Cr} values. There are also presented individual values of the factor M of the THb_{CO} formula obtained by substituting the observed THb_{Cr} value for corresponding THb_{CO} value in the formula.

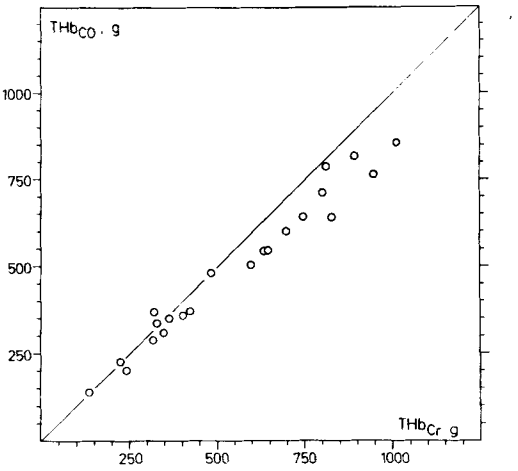

Fig. 1. Total haemoglobin estimated with the alveolar CO- and the ^{51}Cr-method.

The THb_{CO} values of 10 patients were discarded for methodological reasons (cf. Methods). The range of ages of the 22 remaining (12 female, 10 male) patients was 27–84 with a mean of 53 years. In these 22 patients the range of THb_{Cr} was 133–1 009 g. The relationship between the individual values of THb estimated with the alveolar CO method and the ^{51}Cr method is shown in Fig. 1. The simple linear regression of this relationship follows the equations $THb_{CO}=47+0.81\times THb_{Cr}$ and $THb_{Cr}=-40+1.20\times THb_{CO}$, with a correlation coefficient of +0.98. Essentially the same results were obtained on the total material of 32 patients with the follwing regression equation: $THb_{CO}=59+0.77\times THb_{Cr}$ (correlation coefficient + 0.96). Thus with increasing THb, successively lower values were obtained with the alveolar CO method as compared with the ^{51}Cr method and at high levels the differences was about 15%. When no correction for extravascular CO binding was made, the regression equation for the selected material was $THb_{CO}=50+0.85\times THb_{Cr}$ ($r=0.985$) and when 0.5 g Hb/kg body weight was subtracted as extravascular, the equation was $THb_{CO}=16+0.84\times THb_{Cr}$ ($r=0.980$). The error of the intercept (when $THb_{Cr}=0$) was essentially the same (39.8 and 45.1) as in the original equation $THb_{CO}=47+0.81\times THb_{Cr}$ (37.8).

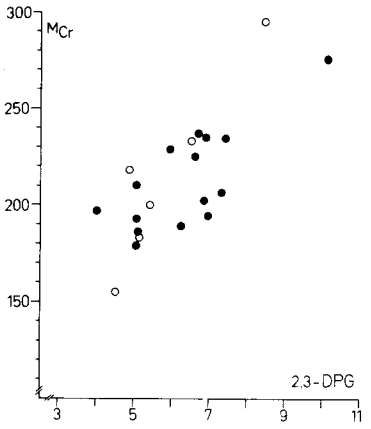

Fig. 2. Correlation between individually calculated M values and the red cell content of 2.3-DPG. The filled symbols refer to the selected group of patients. The DPG concentration is expressed as mMoles per litre of packed red cells.

There was a strong positive correlation ($r=0.757$, $n=15$, $0.001<p<0.005$) in the selected group of patients between the individually calculated M values and the erythrocyte content of 2.3-DPG as shown in Fig. 2. There was a significant correlation ($r=-0.773$, $n=15$, $p<0.001$) between M values and the Hb concentration of venous blood. The relationships were essentially the same for the entire group of patients in which erythrocyte 2.3-DPG was determined ($r=0.774$, $n=21$, $p<0.001$; $r=-0.757$, $n=21$, $p<0.001$). The mean M value for the selected group of 22 patients was 223 (S.D.=24) when no correction for extravascular CO binding was made and 212 (S.D.=23) when the THb_{CO} value was reduced by 5% according to the standard formula. From the regression equations $M=261-0.302\times Hb$ and $M=248-0.285\times Hb$, M values corresponding to a venous Hb concentration of 140 g/l were calculated and were 219 without correction for extravascular CO binding and 208 with the usual correction of 5%.

DISCUSSION

In the present study a close correlation was found between THb estimations performed with the alveolar CO and the ^{51}Cr methods. The results, however, were not identical. At high levels of THb the values obtained using the ^{51}Cr method were about 15% larger than those obtained using the alveolar CO method. These results do not seem to agree with previous investigations (31, 45). When discussing the discrepancy between the results of the two methods it is essential to realize that the haemoglobin mass measured by the two methods are not theoretically identical. The alveolar CO method measures, at least from a theoretical point of view the amount of active haemoglobin and other CO binding haemoproteins in the body excluding inactive haemoglobin, e.g. methaemoglobin. Inactive haemoglobins usually constitute about 0.2–1% of the haemoglobin in the circulating erythrocytes of normal individuals (4, 42). The method utilizing a dilution of ^{51}Cr-tagged erythrocytes measures the amount of cyan-met-reacting haemoglobin in circulating erythrocytes. The presence of inactive haemoglobins will decrease the THb_{CO} value in proportion to their concentration. The concentration of methaemoglobin was not determined in the present study, but there were no reasons to suspect an increased concentration in any of the patients. The influence of extravascular CO binding haemoproteins will be discussed below.

The precision and validity of the ^{51}Cr method was controlled as far as possible. Using this technique the amount of non-erythrocyte bound ^{51}Cr is less than 0.3% (6). In all cases it was possible to ascertain an intravenous location of the injection needle before, during and after the injection of the labelled erythrocytes. The mixing of the tracer was controlled. In order to compare the results of the ^{51}Cr measurements of the present study with the results from another laboratory, the individual values of RCV of all patients were calculated (Table 1*a* and *b*) and the relationship between RCV/kg body weight and venous haematocrit was analysed. The same relationship was analysed on the values of 176 patients without a major degree of splenomegaly and within the same range of venous haematocrit studied by Huber, Lewis & Szur (26)[1] who used a ^{51}Cr method for RCV estimation and a microcapillary centrifuge for haematocrit determination. The relationship between RCV/kg and venous haematocrit in these two investigations are shown in Fig. 3. The results agree well and do not indicate that RCV (or THb) is overestimated with the ^{51}Cr method used in the present study as compared with the method used in London by Huber et al.

[1] The individual values of these patients were kindly offered by these authors for comparison with the present data.

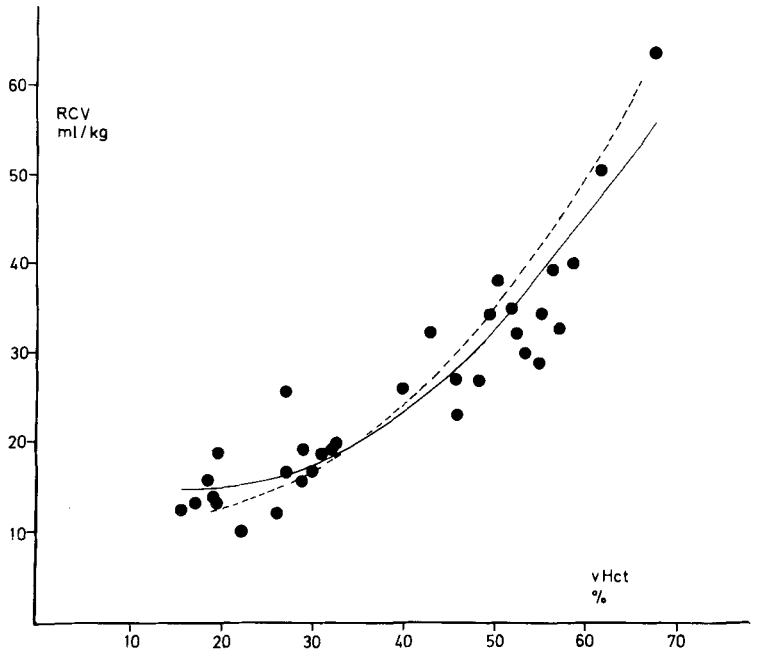

Fig. 3. Correlation between circulating red cell volume/kg body weight and venous haematocrit in 33 patients included in the present study. The corresponding second-degree polynomial regression line ($y=20.4-0.61x+0.017x^2$) is represented by the continuous line. The broken line refers to the second-degree polynomial ($y=13.4-0.37x+0.016x^2$) of the same relationship in 176 patients studied by Huber, Lewis & Szur (26).

The details of the alveolar CO method were also carefully controlled. The methods used for analysis of O_2 and CO concentrations were checked against other methods and other laboratories. The volume of the rebreathing apparatus is estimated by the manufacturer. The residual volume of the subject examined is set to 1.5 litres. Neither of these two volumes may be expected to vary more than one litre and will not influence the results more than 0.3%. For the same reason, a variation of the volume of the rebreathing bags cannot be responsible for our findings. In his report of 1948, Sjöstrand used two 7 litre rubber bags. In more recent publications most authors using the same apparatus as in the present study have calculated with a volume of approximately 5 litres (25) of the rebreathing bags after maximal expiration. As we used bags having a capacity of 6.5 litres (as stated by the manufacturer) this value was used in our calculations. Later we checked the volume after maximal expiration and obtained a mean value of 5.5 litres. Using the value of 6.5 instead of 5.5 litres an underestimation of THb_{CO} of 0.3% is introduced at high as well as at low levels. In earlier studies concerning methods of THb_{CO} estimations the value of 1.34 ml/g Hb has been used as the oxygen binding capacity of haemoglobin. This figure was based upon an assumed haemoglobin molecular weight of 66400. In the present study the value 1.39 ml/g has been used. This is the value calculated from the now accepted molecular weight of haemoglobin which is 64458 (13, 27).

It can be argued that 1.39 ml/g is not a correct value of oxygen binding capacity of haemoglobin *in vivo*, which varies with the amount of inactive haemoglobin. The use of the factor 1.39 instead of 1.34 will reduce the estimate of THb_{CO} with approximately 3.7% at high as well as low levels of THb. The THb_{Cr} will be reduced by the same factor as a consequence of the coefficient of extinction calculated from the new molecular weight of haemoglobin. Consequently the modification of the factor cannot explain our findings of a discrepancy between the two methods.

The effect of an extended CO equilibration time was not studied. To influence the estimation of THb_{CO} significantly, the equilibration time would probably have to be more than doubled. With the present type of apparatus, rebreathing periods of one hour or more seem highly impracticable. Assuming an incomplete mixing of CO within the haemoglobin pool also necessitates an even more incomplete equilibration of this tracer within its extravascular volume of distribution. A prolonged equilibration time would thus increase the amount of CO distributed within this extravascular pool. With extended rebreathing periods, endogenous production of CO from decomposition of hae-

moglobin would also introduce an error varying between individuals. Since the polycythaemia in the cases studied was not caused by pulmonary disease, it seems unlikely that an insufficient CO equilibration could explain a greater difference between the results of the two methods at high than at low levels of THb.

The two remaining details of the CO formula to be discussed are the correction factor for extravascular binding of carbon monoxide and the so-called *M*-factor. Most investigators using the CO method for determination of THb seem to accept the hypothesis that a minor fraction of the CO injected into a rebreathing system during the period of equilibration reacts with myoglobin and other extravascular haemoproteins. The concept of such rather rapidly equilibrating extravascular CO pool is based upon the following observations.

(*a*) Sjöstrand studied the alveolar CO concentration after a single injection of CO into a rebreathing system by taking gas samples every 15 minutes (38). The difference in CO content between the first and second value after the administration of CO was larger than between the subsequent values which as a rule showed a steady decrease. As an explanation, Sjöstrand suggested that the final equilibrium between the CO concentration of the blood and the myoglobin is not reached in 15, but in 30 min. An alternative explanation, also suggested by Sjöstrand, is the removal of CO from the system by the comparatively large gas samples (7 l). This might also explain the tendency of the alveolar CO concentration to decrease during the entire period of study (60 min) in spite of the endogenous CO production.

(*b*) Several series of simultaneous determinations of RCV by CO and different radioactive red cell labels have indicated that CO yields a 5–23 % larger RCV value than determination by the radioactive labels (21, 31, 33, 34, 44). Luomanmäki introduced the term of "extravascular CO capacity" defined as the difference between the total body CO capacity and the intravascular CO capacity (33). For all CO dilution techniques not utilizing radioactive carbon isotopes the availability of an exact method for the determination of COHb concentration is essential. The possibility of estimating COHb by analysis of alveolar gas will be discussed in connection with the M factor. Other methods used in studies comparing CO with dye or radioactive isotope dilution techniques are based upon the determination of the amount of CO which can be released from haemolysed blood by adding ferricyanide or sulphuric acid. The methods are as a rule standardized with scrupulous care regarding the estimation of CO concentration. The accuracy of the estimation of the true COHb concentration is, however, much more difficult to ascertain. An example of such methodological difficulties is given by the discrepancy between the findings of Allen & Root (1), Joels & Pugh (28) and Rodkey, O'Neal & Collison (35) regarding the effect of blood pH on COHb dissociation.

(*c*) Wennesland et al. (44) administered CO gas and ^{51}Cr-labelled red cells to dogs and rabbits and demonstrated an excess of CO in the muscles which could not be explained by CO in the haemoglobin of the blood vessels of the muscles. They remarked that they used "relatively larger doses of CO ... than are used in measurements of blood volume" without reporting the range of COHb concentration obtained. Nor did they report any control experiments in which tissue homogenates were analysed for CO in animals not pre-treated with CO inhalation. With their technique, pepsin powder in 1 N sulphuric acid was added to the tissue homogenates and "six to eight hours were needed for complete liberation of the gas and reduction of an equivalent amount of $PdCl_2$", (44). Sjöstrand has shown that myoglobin under certain circumstances can be decomposed analogously with haemoglobin under formation of CO (39). In such a complex system, as is made by a mixture of tissue homogenate and pepsin–sulphuric acid, the appearance of a palladium–chloride reducing agent is not necessarily evidence of the presence of exchangeable CO in the intact tissue especially if the observation is not referred to adequate control experiments. In a number of experiments Luomanmäki (33) studied the kinetics of the extravascular CO pool in dogs. It should be observed that Luomanmäki in his experiments calculated a mean extravascular CO capacity of 22.9 % (range 14.6–37.5 %) of total body CO capacity. These values are considerably larger than those reported by other investigators. (i) When ^{14}CO was injected into the rebreathing system and ^{51}Cr-tagged erythrocytes into the circulation of a dog simultaneously, the shapes of the blood curves for ^{14}C and ^{51}Cr were identical during the first 60 minutes. Although this finding might be consistent with an extremely rapid equilibration (as concluded by the author) a more plausible explanation would

be a very slow equilibration between the intra- and extravascular CO pools. (ii) When the P_{O_2} of the rebreathing system was varied within the range of 25–92 kPa the steady state concentration of COHb and ^{14}C of blood in the equilibrated system remained the same. (iii) Similarly, when CO was continuously injected into the rebreathing system there was a strictly linear relationship between the volume of CO injected and the COHb concentration of blood in the range of 1–40% COHb. Similar observations were made by Sjöstrand studying the alveolar concentration during intermittent addition of CO (37).

The observations, that variations in P_{O_2} did not influence the equilibrium, rather speak against a rapidly equilibrating extravascular CO pool because of the quite different shapes of the CO dissociation curves for haemoglobin and myoglobin and the unequal *M* values of the two proteins. Further arguments against the existence of a rapidly equilibrating CO pool are given by observations made by Blackmore (5). He studied the distribution of CO in erythrocytes of persons exposed to high concentrations of CO gas for short periods using a staining technique. His observations suggest that after inhalation of CO only the erythrocytes exposed to the gas in the lungs will react with CO and that redistribution of the COHb between the erythrocytes during subsequent circulation does not occur. If, as suggested by his observations, this redistribution between erythrocytes is slow and incomplete, redistribution between erythrocyte haemoglobin and muscular myoglobin with less affinity for CO should be practically none.

We do not pretend to form an opinion on whether a rapidly equilibrating extravascular CO pool exists or not. We believe, however, that the evidence for the existence of such a pool is not particularly well established.

Even if the existence of a rapidly equilibrating extravascular CO pool is plausible, the assumption that this pool is in all individuals equivalent to a constant fraction of the THb is not probable. In a patient with polycythaemia the total myoglobin should be expected to correspond to a smaller fraction of the THb than in a patient with anaemia. If a constant factor of 0.95 is used this may consequently lead to a relative underestimation of the total haemoglobin in polycythaemia and to a relative overestimation in anaemia. The maximal error that reasonably can be introduced by this correction constant is, however, too small to explain the difference between the two methods studied by us.

The alveolar CO method for determinations of THb is finally based on the assumption that COHb concentration can be estimated from the alveolar tension of O_2 and CO according to Haldane's first principle:

$$\frac{COHb}{O_2Hb} = M \frac{pCO}{pO_2},$$

and that the *M* factor is known and does not vary significantly between individuals. Different investigators have used different values of *M*. In his early works, Sjöstrand used a value of 210, originally determined *in vitro* by Sendroy, Liu & van Slyke (36). (Other investigators using *in vitro* techniques have reported values of the same order of magnitude.) Carlsten, Holmgren, Linroth, Sjöstrand & Ström (7) comparing different methods for the estimation of COHb concentration, found varying values of *M*. For their main group of experiments the mean value was 245. It was later recalculated to 231 by Wiklander (45) from the original data of Carlsten et al., and was in moderate agreement to the values calculated from results published by Forbes, Sargent & Roughton (17). The value of 231 has afterwards mostly been used in Sweden. This *M* value was described as "empirical" by Linderholm (32) and by Cederlund, Holmgren & Håkansson (9). Katchman, Murphy, Offner & Fox (29) analysed alveolar gas and venous blood simultaneously with infrared technique and found a mean *M* value of 219 (range 167–277). In our series of experiments we found a mean *M* value of 212 if correction for extravascular CO capacity was made and 218 if no correction was made. A positive correlation was found between the individually calculated *M* values and the intra-erythrocytic 2.3-DPG concentrations and a negative correlation between the *M* value and haemoglobin concentration. Since there is a known relationship between the red cell content of 2.3-DPG and haemoglobin concentration at least in the range of normal and decreased haemoglobin concentrations (23, 24), the available data do not permit any conclusions regarding causality. Engel, Rodkey, O'Neal & Collison (15) have published observations suggesting that the ratio of the affinities of CO and O_2 is less for haemoglobin F than for haemoglobin A. At a fixed content of 2.3-DPG, fetal red cells have a higher

affinity for O_2 than adult red cells since haemoglobin F has a smaller affinity for 2.3-DPG than haemoglobin A. It is therefore reasonable to assume that the affinity of haemoglobin F and haemoglobin A with respect to the relative affinities for CO and O_2 may be interpreted to show that 2.3-DPG decreases the affinity for CO less than for O_2. The correlation found in the present study may be taken as good support for this idea.

Further support for this theory has recently been offered by the finding of Lawson (30), that the initial rate of displacement of O_2 by CO in intact red cells from normal subjects is lower than that in red cells from subjects with anaemia due to blood loss.

Wiklander, using similar techniques on a similar group of patients as in the present study, found good agreement between total haemoglobin determinations made with the alveolar CO method and with ^{32}P-labelled erythrocytes (45). Thus the disagreement between the studies has to be explained.

(1) Wiklander introduced a value of M, which was based on a material of normal persons and later on described as "empirical" (9, 32). The introduction of a corresponding empirical factor in the calculations of the present study would obviously create a better agreement between the two methods.

(2) The group of patients in Wiklander's study represent a more narrow distribution of total haemoglobin values (mean 502 g, S.D. 120 g) than the patients of the present study (mean 550 g, S.D. 262 g). Since the difference between the values obtained with the two methods of the present study is correlated with the total body haemoglobin value, the possibilities of revealing a discrepancy between the methods should be facilitated by a wider range of distribution.

(3) Fifty per cent of the patients studied by Wiklander had initial COHb values of more than 1.0%, in the majority of cases probably as an effect of smoking. The "smokers" were, however, skewly distributed within the distribution of total haemoglobin values (cases with initial COHb less than 1.0% had a mean THb of 453 g, S.D. 130 g and cases with initial COHb 1.0% or more a mean THb of 551 g, S.D. 108 g), and may hypothetically be regarded as a population different from the "non-smokers". The regression equations that can be calculated from Wiklander's figures are $THb_{CO}=1+0.99\times THb_p$ for the "non-smokers" and $THb_{CO}=74+0.87\times THb_p$ for the "smokers".

Engstedt, Peric & Tribukait (16) made simultaneous estimations of total haemoglobin with ^{51}Cr and alveolar CO methods in anaemic and polycythaemic mice. They found higher values with the CO method than with the ^{51}Cr tagging technique. The relative difference between the values obtained with the two methods was about the same in the two groups of animals. It should be observed that the mice were made polycythaemic by prolonged exposure to decreased atmospheric pressure. Under these experimental conditions, the erythrocyte content of 2.3-DPG may have been increased in the polycythaemic as well as in the anaemic animals. Thus their observations are not inconsistent with the hypothesis that intraerythrocyte 2.3-DPG may influence the value of the M-factor. This question is however best elucidated by an *in vitro* experiment.

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Received May 20, 1977

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