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THE OXYGEN AND CARBON MONOXIDE CAPACITIES OF FOETAL AND ADULT BLOOD

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SUMMARY

1. The oxygen capacities of foetal and adult blood have been compared by direct measurement of their oxygen and carbon monoxide combining power. Corrections have been applied to the capacities obtained from oxygen content determinations for the presence of carboxyhaemoglobin and methaemoglobin.

2. Mean values for the capacities of foetal blood derived from oxygen and carbon monoxide content were 1·312 ml./g (S.E. ± 0·007) and 1·331 (S.E. ± 0·002) respectively, while those for adult blood were 1·306 ml./g (S.E. ± 0·006) and 1·331 (S.E. ± 0·0005) respectively.

3. For neither carbon monoxide nor oxygen was there a significant difference ($P > 0·05$) of capacity between adult and foetal blood. There was, however, a significant difference ($P < 0·05$) on comparing the two methods of measurement on each form of blood and values from both methods were significantly different ($P < 0·001$) from the theoretical value of 1·39 ml. oxygen/g haemoglobin derived from the molecular weight of haemoglobin.

4. These results suggest that the International Cyanmethaemoglobin Standard gives values of haemoglobin concentration which are approximately 6% higher than levels indicated by oxygen combining power.

INTRODUCTION

The oxygen content of blood may either be measured directly or derived from the oxygen capacity of haemoglobin. The difficulties associated with the direct methods of measurement have resulted in the frequent use of the oxygen capacity in the determinations of content which are required in a wide range of cardio-respiratory investigations.

In adult blood, recent investigators have found values between 1·30 ml./g (Theye, 1970) and 1·37 (Scherrer, Kung & Mösli, 1971), while the theoretical

value of the oxygen capacity calculated from the molecular weight of adult haemoglobin of 64,458·5 (Braunitzer, 1963) is 1·39 ml. oxygen/g haemoglobin.

The few published studies of the oxygen capacity of foetal blood have given even more widely differing values. Thus Swierczewski & Minkowski (1956) found values for the capacity of arterial foetal blood between 0·99–1·67 ml. oxygen/g haemoglobin, with a similar range of results being obtained by Goodlin & Kaiser (1957).

Since oxygen and carbon monoxide combine in the same molar proportions with haemoglobin, 'oxygen' capacity may be determined using either gas; the classical work on oxygen capacity by Hüfner (1894) was based on carbon monoxide content, as were the measurements on adult blood (King, Gilchrist, Wootton, O'Brien, Jope, Quelch, Peterson, Strangeways & Ramsey, 1948; Remmer, 1956). However, no such determinations have been found for foetal blood.

In view of the disparity of previous results, an attempt has been made to compare the oxygen capacities of foetal and adult blood by determination of their oxygen and carbon monoxide combining powers.

METHODS

Measurements of oxygen capacity were carried out by two separate methods which determined respectively the oxygen and carbon monoxide combining power. Foetal blood samples (20 ml.) were obtained from the placental vessels immediately post partum and stored in syringes containing 0·07 ml. heparin (5000 i.u./ml.) at 4° C until required, the maximum time between withdrawal of the sample and the first analysis being 12 hr. Venous blood (25 ml.) was taken from one non-smoking adult control subject at the start of each day and similarly stored at 4° C. The same adult donor was used throughout these studies. Starch gel electrophoresis of blood from this subject showed no abnormal haemoglobins.

1. *Determination of oxygen combining power*

Measurements were made on fresh samples of foetal and adult blood on 4 separate days. On a single day, alternate 5 ml. aliquots of the same foetal and adult blood samples were equilibrated with 5% carbon dioxide in oxygen in glass tonometers (Adams & Morgan-Hughes, 1967), the water jackets of which were maintained at 37° C by a circulating water-bath. A total of four foetal and four adult samples were analysed on each day. The gasses were humidified and the rate of flow controlled at approximately 2·5 ml./min for 30–40 min. At the end of this period two 2 ml. syringes were filled with blood. From the first of these an Ostwald pipette was immediately filled and a 1 ml. sample introduced into the Van Slyke chamber. The remainder of the blood was used for the determination of haemoglobin concentration. From the second syringe the P_{O_2}, P_{CO_2} and pH were measured with electrodes. The Clark oxygen electrode (Radiometer) was calibrated with oxygen-free nitrogen (British Oxygen Company) and with blood equilibrated with 100% oxygen.

The oxygen content was measured with the Van Slyke manometric apparatus (Gallenkamp Model MC 500). The technique used was that described by Bartels,

Bücherl, Hertz, Rodewald & Schwab (1963) with the exception that the blood-saponin-ferricyanide mixture was stirred for 5 min instead of being shaken. Calibration with weighed mercury of the 2 ml. volume in the upper part of the Van Slyke chamber and the 1 ml. Ostwald pipette used throughout this study gave values of 1·995 and 1·003 ml. respectively.

The haemoglobin concentration was measured by the cyanmethaemoglobin method with optical densities read at 540 nm with a Pye Unicam SP 1800 spectrophotometer. From the blood remaining in the first syringe of each analysis, four dilutions (1:201) were made in freshly prepared Drabkin solution (Aculute, Ortho Pharmaceuticals) using an autodilutor (Hook and Tucker). Four similar dilutions were made of a lysed whole blood standard (Diagnostic Reagents). Calibration of the autodilutor by comparison with manual methods was carried out and the diluted lysed whole blood standard was compared with the International Cyanmethaemoglobin Standard (Eilers, 1967) at the beginning and end of each day.

The percentage of foetal haemoglobin present in each foetal sample was measured on each day by the method of alkali denaturation (Betke, Marti & Schlicht, 1959). Carboxyhaemoglobin was determined in duplicate for the foetal and adult blood on each day, samples being taken after the first period of equilibration, using the method of Commins & Lawther (1965). Methaemoglobin was measured in duplicate on the foetal blood daily by the method of Evelyn & Malloy (1938). The value of the methaemoglobin concentration of the adult blood was taken from the mean obtained on twelve consecutive days at the end of the study, using the same method.

2. *Determination of carbon monoxide combining power*

Measurements on the foetal and adult blood were carried out on different days. Each type of blood was examined twice on 4 consecutive days, a fresh sample being used on each. The samples were obtained in the manner described for oxygen combining power.

The carbon monoxide content was determined with the same apparatus and Ostwald pipette as in the previous part of the study, using the method of Van Slyke, Hiller, Weisiger & Cruz (1946). Three modifications were made to this method in that the equilibration time with carbon monoxide was increased to three minutes, that for the release of carbon monoxide from carboxyhaemoglobin was increased to 4 min, and an Ostwald pipette replaced the 1 ml. blow-out pipette described in the original method.

The technique involves the introduction of a blood sample into the analysis chamber, reduction of any methaemoglobin present with sodium dithionite and equilibration with carbon monoxide. The unbound carbon monoxide and nitrogen are then extracted from the solution, carbon monoxide and dioxide are released from the blood, the latter being absorbed with sodium hydroxide, and the carbon monoxide remaining is measured.

The haemoglobin concentration of each sample was again determined by the cyanmethaemoglobin method as in the measurement of oxygen combining power. However, dilution in this part of the study was carried out manually in duplicate, 0·5 ml. of the blood remaining in the syringe after the determinations of carbon monoxide content was pipetted into 100 ml. of freshly prepared Drabkin solution in a certified volumetric flask. The optical density of the solution was read after 20 min against the International Cyanmethaemoglobin Standard.

RESULTS

The mean values found for the oxygen capacity when determined by oxygen combining power are shown in Table 1. On Day 1, a significant difference ($P < 0{\cdot}05$) was found between the mean values obtained for each type of blood. The low mean value for the oxygen capacity of foetal blood of 1·260 ml./g on that day was associated with a high level of carboxyhaemoglobin. Correction of the haemoglobin concentrations measured by the cyanmethaemoglobin method for the presence of methaemoglobin and carboxyhaemoglobin which do not combine with oxygen, raised the values initially determined for the oxygen capacity to the corrected values also shown in Table 1.

TABLE 1. Shows the oxygen capacity (with 1 S.D.) obtained in foetal and adult blood by direct measurement of oxygen content and after correction for the percentage of methaemoglobin and carboxyhaemoglobin. Each sample of blood was analysed four times on each day. The percentage of foetal haemoglobin found in each foetal sample is also shown. The adult value for methaemoglobin is the mean of twenty-four measurements made on twelve consecutive days at the end of the study

	Foetal					Adult			
Day	O_2 capacity (measured)	% HbF	% HbCO	% HbMet	O_2 capacity (corrected)	O_2 capacity (measured)	% HbCO	% HbMet	O_2 capacity (corrected)
1	1·260 (0·012)	54	3·5	0·83	1·315	1·303 (0·007)	0·32	0·40	1·313
2	1·303 (0·006)	63	1·14	0·75	1·330	1·305 (0·011)	0·30	0·40	1·314
3	1·273 (0·008)	50	0·64	1·78	1·304	1·280 (0·014)	0·23	0·40	1·288
4	1·273 (0·011)	77	0·41	1·68	1·300	1·299 (0·020)	0·41	0·40	1·310
Mean	—	—	—	—	1·312	—	—	—	1·306
S.E.	—	—	—	—	0·007	—	—	—	0·006

The mean value for the methaemoglobin in the adult subject determined in duplicate on 12 consecutive days, was 0·40 % (S.E. ± 0·05). An analysis of variance of these measurements showed that the between day variation was not significantly different ($P > 0{\cdot}05$) from that found within days.

The results for the capacity when determined with carbon monoxide (Tables 2 and 3) show daily mean values which are approximately 1·5 % higher than those found with oxygen. Similarly the variation of values within and between days is less. The percentages of foetal haemoglobin

TABLE 2. Shows the carbon monoxide content, haemoglobin concentration and 'oxygen' capacity for the duplicate measurements on samples of foetal blood on 4 consecutive days. The percentage of foetal haemoglobin found in each sample is also shown

	CO content (vol./100 ml.)		Haemoglobin (g/100 ml.)		'Oxygen' capacity	%
Day	1	2	1	2	(vol/g Hb)	foetal Hb
5	17·66	17·67	13·32	13·29	1·328	62
6	22·47	22·82	17·03	16·97	1·332	58
7	18·42	18·41	13·86	13·86	1·329	53
8	19·58	19·43	14·60	14·62	1·335	59
				Mean	1·331	
				S.E. ±	0·002	

TABLE 3. Shows the carbon monoxide content, haemoglobin concentration and 'oxygen' capacity for the duplicate measurements made on adult blood samples on 4 consecutive days

	CO content (vol./100 ml.)		Haemoglobin (g/100 ml.)		'Oxygen' capacity
Day					(vol./g Hb)
9	20·72	20·62	15·52	15·52	1·332
10	20·72	20·81	15·61	15·60	1·331
11	19·59	19·56	14·71	14·72	1·330
12	20·24	20·06	15·15	15·09	1·333
				Mean	1·331
				S.E. ±	0·0005

TABLE 4. Shows the mean and S.D. found in repeated measurements of each variable and the individual effect of 1 S.D. on the oxygen capacity. The Van Slyke data is based on determinations of carbon monoxide content

	Haemoglobin (g/100 ml.)			
Variable	Autodilution	manual	P_{O_2} (mmHg)	Van Slyke (vol./100 ml.)
Mean	14·30	14·97	676·0	20·00
n	30	16	20	16
S.D.	0·09	0·02	8·9	0·11
Effect of 1 S.D. on oxygen capacity (ml./g)	0·008	0·002	0·002	0·007

found in the foetal samples in the two studies show no significant difference ($P > 0·05$) (Tables 1 and 2).

The random error shown in repeated measurements of the different variables is shown in Table 4. The effect that 1 S.D. of these measurements would have individually on the oxygen capacity is also shown. The latter

values illustrate that of the three variables that were measured in each determination of the oxygen capacity, the effect on the capacity of 1 S.D. of the measurements of haemoglobin concentration would be four times as great as that found for the determinations of oxygen tension but similar to those of oxygen content.

DISCUSSION

Previous investigations of the oxygen capacity of foetal haemoglobin have been based on measurements of oxygen combining power only. In an analysis of fifty-eight foetal samples, Swierczewski & Minkowski (1956) found a mean value of 1·26 ml./g (S.D. ± 0·18) in comparison with 1·34 ml./g (S.D. ± 0·08) for sixteen adult controls. They assumed an iron content of haemoglobin of 0·34%, which gave a theoretical oxygen capacity of 1·36 ml./g. Kirschbaum (1964) assumed a capacity for adult blood of 1·34 ml./g and showed no difference between this value and the mean of estimations on fifty-three foetal samples (range 1·22–1·40 ml./g).

In neither of these studies were any measurements of methaemoglobin or carboxyhaemoglobin made, and the low foetal values may be explained by the high levels of foetal carboxyhaemoglobin of up to 7·6% which may be present (Longo, 1970). The values for oxygen capacity of adult blood reported in the present paper are within the range of those found in recent studies (Table 5). In a comparison of the *in vivo* and *in vitro* oxygen capacities in this and one other adult subject, there was no significant difference in the mean values obtained (Gregory & Millar, 1973).

In the present study no significant difference ($P > 0{\cdot}05$) was found between the mean values of oxygen capacity of foetal and adult blood when determined either with oxygen or with carbon monoxide. A significant difference ($P < 0{\cdot}05$) was, however, found between the mean values obtained with each type of blood when using the two methods. All these results were significantly different ($P < 0{\cdot}001$) from the theoretical value of 1·39 ml./g for adult blood.

In this study the P_{O_2} at which the blood was equilibrated was in the region of 650 mmHg, and 100% saturation was therefore assumed (Nahas, Morgan & Wood, 1952; Severinghaus, Roughton & Bradley, 1972). A Bunsen solubility coefficient of oxygen in blood at 37° C of 0·0239 was used (Sendroy, Dillon & Van Slyke, 1934; Severinghaus *et al.* 1972). The mean value for methaemoglobin found in the adult subject is in agreement with those found by Van Slyke *et al.* (1946) of 0·4% and Paul & Kemp (1944) of 0·6%. Similarly the values found for methaemoglobin in the foetal samples fall within the range of 0–2·8% reported by Kravitz, Elegant, Kaiser & Kagan (1956) in new-borns.

To measure the oxygen capacity of haemoglobin using carbon monoxide, no correction need be applied for any carboxyhaemoglobin initially present and any methaemoglobin is converted to reduced haemoglobin by sodium dithionite before saturation with carbon monoxide. The greater reproducibility of the measurements of capacity with carbon monoxide can be seen from the results of the two methods (Tables 1, 2, 3). Thus the over-all range of the values for oxygen and carbon monoxide capacity during this study for the adult blood was 0·026 and 0·003 ml./g respectively.

TABLE 5. Summarizes the recent values that have been obtained for the oxygen capacity of adult blood

	Oxygen capacity (ml./g)
Warrell & Pope (1969)	1·33
Föex, Prys-Roberts, Hahn & Fenton (1970)	1·33
Theye (1970)	1·30
Bursaux, Dubos & Poyart (1971)	1·31
Scherrer, Küng & Mösli (1971)	1·37

Since the oxygen and carbon monoxide capacities for the adult blood which are significantly different at the 5 % level were not measured on the same day, the difference between their respective over-all means of 1·306 and 1·331 could be accounted for by 'between day' variation. However, an analysis of variance of these measurements of capacity with oxygen has shown no significant difference at the 5 % level, and thus suggests that the difference is not due to 'between day' variation.

After correction for methaemoglobin and carboxyhaemoglobin a significant difference remained between the capacities when measured with oxygen and carbon monoxide in both foetal and adult blood. This may be explained by the findings of Conant, Scott & Douglas (1928) that haemochromogens will combine with carbon monoxide.

The Van Slyke manometric apparatus and Ostwald pipette that were used throughout this study have been calibrated using solutions of hydrogen peroxide (Gregory, 1973). The mean difference between the manometric and titrimetric measurements was 0·07 ml. oxygen/100 ml. solution (S.E. $\pm$ 0·03, n.s.). No correction was made for this error in the present paper.

The haemoglobin concentrations were measured using the International Cyanmethaemoglobin Standard (Eilers, 1967). The concentration of the standard which is based on a millimolar extinction coefficient of cyanmethaemoglobin (HiCN) of 44·0, was obtained from the data of Zijlstra & Van Kampen (1960) who found a value of 11·05 (S.D. $\pm$ 0·16) based on an equivalent weight of haemoglobin of 16·114. A previous value of 11·5 had been accepted in 1958 (Cannan). As Zijlstra *et al.* (1960) have noted, a

problem arises in this measurement from the lack of any direct method of measuring HiCN concentration other than photometrically.

TABLE 6. The difference between the theoretical combining power based on iron determinations (55·9 mg Fe = 22·4 ml. O_2) and determinations of oxygen and carbon monoxide combining power measured with the Van Slyke apparatus. The present paper and that of Zijlstra *et al.* (1964) are based on measurements of haemoglobin concentration using the International Cyanmethaemoglobin Standard

Author	Difference from titrimetric iron determination (%)	
	CO combining power	O_2 combining power
Macfarlane *et al.* (1944)	—	−0·01
King *et al.* (1948)	−1·1	−2·5
Remmer (1956)	−1·6	−2·4
Zijlstra *et al.* (1960)	−2·4	—
Zijlstra *et al.* (1964)	—	−5·7
This paper	−4·3	−5·8

The oxygen and carbon monoxide combining power of blood has been compared with titrimetric iron concentrations and with the International Standard (Table 6). The present paper and that of Zijlstra, van Assendelft & Rijskamp (1964) which were dependent on the International Cyanmethaemoglobin Standard for their measurements of haemoglobin concentration show lower oxygen capacities than those found by King *et al.* (1948) who compared iron concentrations and capacities directly without recourse to the extinction coefficient of cyanmethaemoglobin or molecular weight of haemoglobin. The difference between the theoretical capacity and that determined with carbon monoxide is also greater than that previously found.

The International Standard is based ultimately on the measurement of iron, from which the accepted extinction coefficient of 44·0 was derived (Zijlstra *et al.* 1964; Eilers, 1967). However, the inclusion of inactive iron would lead to an underestimate of the true extinction coefficient and the results of both Zijlstra *et al.* (1964) and the present studies (Table 6) suggest that this discrepancy is of the order of 6 % when related to oxygen capacity.

The studies reported in this paper would support an extinction coefficient of 46·6 based on oxygen capacity for both foetal and adult blood. This coefficient would yield the theoretical oxygen capacity of 1·39 ml./g and would result in a general reduction of reported haemoglobin values by 6 % or 0·9 g/100 ml. in normal blood. The use of this extinction coefficient would thereby relate the haemoglobin concentration to the gas combining power which has a greater relevance than iron content.

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