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CARBAMINO COMPOUNDS OF HAEMOGLOBIN IN HUMAN ADULT AND FOETAL BLOOD

By C. BAUER and E. SCHRÖDER

From the Physiologisches Institut der Medizinischen Hochschule, 3 Hannover, Germany

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SUMMARY

1. The carbamate ($HbCO_2$) concentration in oxygenated and deoxygenated human adult and foetal red blood cells was estimated at a constant pressure of carbon dioxide (P_{CO_2} = 40 mm Hg) and various pH values of the serum. The Donnan ratio for chloride and bicarbonate ions was used to calculate the bicarbonate concentration in the red cells. With this figure the carbamate concentration was calculated as follows:

$$[HbCO_2] = [\text{Total } CO_2] - [HCO^-_3] - [\text{dissolved } CO_2].$$

2. At a given pH value in the red cell deoxygenated foetal red cells contain more $HbCO_2$ than deoxygenated adult ones. Upon oxygenation (at constant pH) $HbCO_2$ drops in both types of erythrocytes to lower values than in deoxygenated cells. The fraction of 'oxylabile carbamate' ($-\Delta HbCO_2/\Delta HbO_2$) at a red cell pH of 7·2 and a P_{CO_2} of 40 mm Hg is 0·117 in foetal and 0·081 in adult erythrocytes.

3. From the fraction of moles carbamate formed per Hb monomer (moles CO_2/mole Hb_1) K'_c and K'_z, the apparent carbamate equilibrium constants were calculated which can be used to estimate the carbamate concentration in normal adult and foetal blood.

4. The first apparent dissociation constant of carbonic acid is significantly higher in oxygenated ($-\log_{10}K'_1 = pK'_1 = 6{\cdot}10$) than in deoxygenated ($pK'_1 = 6{\cdot}12$) adult red cells, whereas in foetal red cells the difference is smaller and statistically not significant.

5. For a given set of physiological conditions in arterial and mixed venous blood in respect to oxygen saturation, P_{CO_2} and pH, the fractional contribution of carbamino compounds of haemoglobin to the amount of carbon dioxide which is exchanged during the respiratory cycle was computed on the basis of the present results and found to be 10·5 % in adult and 19 % in foetal blood.

INTRODUCTION

The reaction of carbon dioxide with the α-amino groups of the N-termini of the haemoglobin molecule occurs according to the following reaction scheme:

$$Hb-NH^+_3 \rightleftarrows Hb-NH_2+H^+, \quad (1)$$

$$Hb-NH_2+CO_2 \rightleftarrows Hb-NHCOOH \rightleftarrows Hb-NHCOO^-+H^+. \quad (2)$$

The carbamino compounds formed at the uncharged α-amino groups are at physiological pH values almost totally in the charged form (Hb-$NHCOO^-$) which is then called carbamate.

From a physiological point of view this compound is of interest since it has been shown and generally accepted that about 30% of the total carbon dioxide which is given off or taken up from the blood during the respiratory cycle comprises carbamate (Ferguson, 1936; Rossi-Bernardi & Roughton, 1967). The experiments on which this conclusion was based were carried out in dialysed haemoglobin solutions rather than in whole blood, i.e. the assumption was made that haemoglobin has the same functional properties in respect to the binding of carbon dioxide, both in dialysed solutions and in red cells.

In more recent years it has been shown, however, that the much higher oxygen affinity of dialysed haemoglobin solutions in comparison to whole blood can be explained by the loss of certain organic phosphates, in particular 2,3-diphosphoglycerate (2,3-DPG) which occurs in about equimolar concentrations to haemoglobin in the red cells of most mammals (Benesch & Benesch, 1967; Chanutin & Curnish, 1967; Benesch, Benesch & Yu, 1968). Later on it was demonstrated that 2,3-DPG not only decreases the oxygen affinity of haemoglobin but also its carbon dioxide affinity, i.e. the formation of carbamate (Bauer, 1969, 1970; Pace, Rossi-Bernardi & Roughton 1970; Tomita & Riggs, 1971; Siggaard-Andersen, 1971). It thus became evident that the amount of carbamate formed at the haemoglobin molecule not only depends upon pH, P_{CO_2} and oxygen saturation but also on the concentration of organic phosphate esters normally present in the red cell.

All previous attempts to determine the effect of these four variables on the formation of carbamino compounds were carried out in haemoglobin solutions and under different experimental conditions which do not allow estimation of the carbamate concentration in the erythrocytes. For this reason it seemed worth while to estimate the concentration of carbamate in red cells under as nearly physiological conditions as possible to evaluate more closely the contribution of this compound to the carbon dioxide exchange during the respiratory cycle both in adult and foetal human blood.

Part of the material in this paper has been presented in preliminary form (Bauer, 1971).

METHODS

Preparation of blood samples. Adult blood was obtained by venepuncture without stasis, from members of the laboratory. Foetal blood was sampled during normal deliveries from the placental end of the umbilical cord immediately after the cord was cut. In both cases calcium heparinate supplied by a generous gift of the Deutsche Hoffman La Roche Company was used as anticoagulant. In order to change the pH of the blood samples, they were centrifuged and some of the serum replaced by slow addition of an equal volume of 0·15 M-HCl or 0·15 M-NaOH to which 10 μM glucose had been added, the haematocrit thus being kept constant. All samples were equilibrated at 37° C for 100 min in siliconized tonometers described by Laue (1951) with water vapour saturated gas mixtures containing 5·6% CO_2 (P_{CO_2} = 40 mm Hg) in pure nitrogen and pure oxygen respectively. An equilibration time of 100 min was found necessary to reach constancy of the pH value of the samples to which HCl or NaOH was added. Longer equilibrations (up to 130 min) did not change the pH within 0·005 pH units.

The integrity of the red cells in the equilibrated samples was checked by measuring the haemoglobin concentration in the serum using the benzidine method of Crosby & Furth (1956). Usually, the haemoglobin concentration in the serum did not exceed 2–3% of the total. In some instances, however, where higher haemoglobin concentrations in the serum were observed (particularly at high pH values) the corresponding samples were discarded. The concentration of 2,3-DPG in red cells was measured according to Krimsky (1962) and found to decrease in the samples with the lowest pH values by about 20% and in the samples with the highest pH values by 5–10% during the time of equilibration. No obvious difference in the rate of degradation of 2,3-DPG between adult and foetal cells could be detected.

After equilibration serum and cells were separated by centrifugation under liquid paraffin for 15 min at 2000 rev/min and 37° C (S.D. ± 0·5) using a centrifuge equipped with a heating coil and a thermostat (Christ Company, Seesen/Harz) and used as such for further analyses. The mean difference between the pH values of the equilibrated blood samples and the serum after centrifugation at 37° C was ± 0·012 pH units (S.D. ± 0·006) indicating that there was no significant loss of carbon dioxide during the centrifugation procedure.

Analyses. Micromanometric analyses for carbon dioxide and oxygen in both serum and packed erythrocytes were done in duplicate with the apparatus described by van Slyke & Plazin (1961) on 50 μl. samples. The chloride concentration of serum and erythrocytes was measured after deproteinization with 3 N-$HClO_4$ using the Aminco-Cotlove chloride titrator (Aminco Co., Silver Springs, Maryland). In various recovery experiments it was established that the variability between the amounts of chloride added and found was less than 1%. pH was measured using a Radiometer assembly (Radiometer Co., Copenhagen, Denmark) consisting of a pH meter type 4 and a glass electrode type G297/92 which was calibrated with Radiometer precision buffers. For determination of red cell pH, the packed erythrocytes were transferred anaerobically in glass capillaries and lysed in the Radiometer haemolyser. The pH of the lysate was measured with the same type of glass electrode mentioned above. The water concentration of each sample was determined by drying a thin layer of either serum or erythrocytes at 105° C to constant weight. The haemoglobin concentration of blood and packed red cells was determined after conversion to cyanmethaemoglobin. The volume of serum 'trapped' between the erythrocytes was determined using Evans blue as an indicator (Jackson & Nutt, 1951) and was found

to be 3% for both foetal and adult blood. This figure was then used to correct the pertinent values found in packed erythrocytes.

Calculations. The concentration of carbamino compounds in the red cell was calculated from the equation

$$[HbCO_2]_C = [\text{Total } CO_2]_C - [HCO_3^-]_C - [CO_2\text{diss}]_C, \quad (3)$$

whereby $[HbCO_2]_C$ denotes the concentration of carbamino compounds, $[HCO_3^-]_C$ the bicarbonate concentration and $[CO_2\text{diss}]_C$ the concentration of dissolved carbon dioxide, all in m-mole/l. red cells. The concentration of dissolved carbon dioxide was calculated using a solubility coefficient of 0·443 ml. gas/ml. red cells at 760 mm Hg (Bartels, Bücherl, Hertz, Rodewald & Schwab, 1959).

The bicarbonate concentration in the red cells was obtained from the equation

$$[HCO_3^-]_C = r \times [HCO_3^-]_S \times H_2O_C/H_2O_S, \quad (4)$$

the rearranged form of the Donnan distribution ratio for chloride and bicarbonate ions between red cells and serum:

$$\frac{[Cl^-]_C/H_2O_C}{[Cl^-]_S/H_2O_S} = \frac{[HCO_3^-]_C/H_2O_C}{[HCO_3^-]_S/H_2O_S} = r. \quad (5)$$

The bicarbonate concentration in the serum $[HCO_3^-]_S$ was obtained by subtracting the amount of dissolved carbon dioxide from the total carbon dioxide found in the serum using a solubility coefficient of 0·515 ml. gas/ml. serum at 760 mm Hg (Bartels & Wribitzky, 1960). H_2O_C and H_2O_S are the weight fractions of water in erythrocytes and serum and r is the distribution ratio of chloride and bicarbonate ions between the water phase of the serum and the water phase of the red cells. The concentration dimensions in eq. (5) are therefore given as m-mole/l. red cell H_2O. Solving for $[HCO_3^-]_C$ leads then to the bicarbonate concentration in m-mole/l. red cells. The amount of carbamate found in the red cells was related to the concentration of haemoglobin monomers of the actual sample and expressed therefore as moles carbon dioxide/mole haemoglobin monomer (Hb_1).

Statistics. The linear regression equations for the various correlated parameters were calculated by the method of the least squares and the identity of these linear regressions was tested statistically using a programme which is described in detail in Wissenschaftliche Tabellen Geigy (1968). Comparison of the means of paired and unpaired data was done two-tailed with Student's t test. Differences were considered significant at a level of $P < 0{\cdot}05$.

RESULTS

The number of moles carbamate formed per haemoglobin monomer as a function of red cell pH (pH_c) of foetal and adult erythrocytes is shown in Fig. 1 both in the absence and the presence of oxygen. It can be seen that the fraction of carbamate:

(*a*) Is higher in deoxygenated samples than in oxygenated ones when examined at the same pH and P_{CO_2}. This is true both in adult and foetal red blood cells.

(*b*) Increases with increasing pH_c in all samples investigated, and that the difference between the fraction of carbamate formed in deoxygenated and oxygenated cells (called 'oxylabile carbamate' by Rossi-Bernardi & Roughton, 1967) increases with increasing pH_c.

(*c*) Is higher in deoxygenated foetal erythrocytes when compared to the pertinent adult ones, whereas in the presence of oxygen no apparent difference exists between the two types of cells.

The ratio between the hydrogen ion activities in serum and red cells (r_{aH^+})as a function of pH_s is shown in Fig. 2. From the regression equations the intraerythrocytic pH values at pH_s 7·4 are found to be 7·165 and 7·212 in oxygenated and deoxygenated adult erythrocytes respectively; the corresponding figures for foetal erythrocytes are 7·179 and 7·217. The pH difference between oxygenated and deoxygenated cells at pH_s 7·4 and a P_{CO_2} of 40 mm Hg is thus significantly lower ($0{\cdot}025 < P < 0{\cdot}05$) in foetal erythrocytes ($\Delta pH = 0{\cdot}038$) compared to adult ones ($\Delta pH = 0{\cdot}047$).

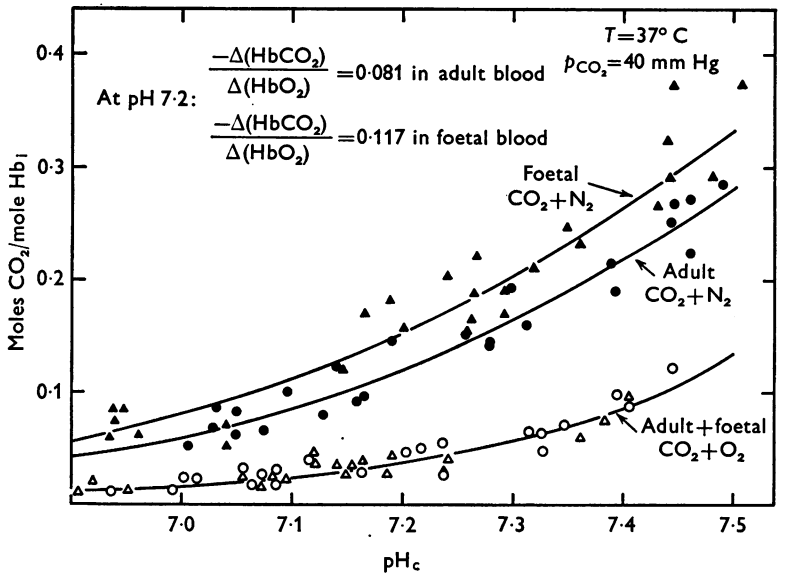

Fig. 1. Plot of moles carbamate formed per mole of haemoglobin monomer (moles CO_2/mole Hb_i) as a function of red cell pH (pH_c) at constant P_{CO_2}. The upper curve (▲) was obtained in deoxygenated foetal red blood cells, the middle curve (●) in deoxygenated adult red blood cells and the lower curve (△, ○) in oxygenated red blood cells of both types. The lines have been calculated using eq. (9) and the constants given in Table 2.

From the calculated bicarbonate concentrations we estimated the apparent first ionization constant of carbonic acid in serum and red cells from the Henderson–Hasselbalch equation:

$$pK_1' = pH - \log \frac{[HCO_3^-]}{\alpha . P_{CO_2}}, \qquad (6)$$

whereby $pK_1' = -\log_{10} K'_1$ and α, the solubility coefficient of carbon dioxide which has the numerical value referred to earlier. The results of these computations based on average figures are given in Table 1. The difference between values of pK_1' for oxygenated and deoxygenated adult erythrocytes was statistically significant ($0{\cdot}025 < P < 0{\cdot}05$) whereas the corresponding figures for foetal red cells were not ($0{\cdot}7 < P < 0{\cdot}8$). There was no significant difference of pK_1' in the serum of either sample.

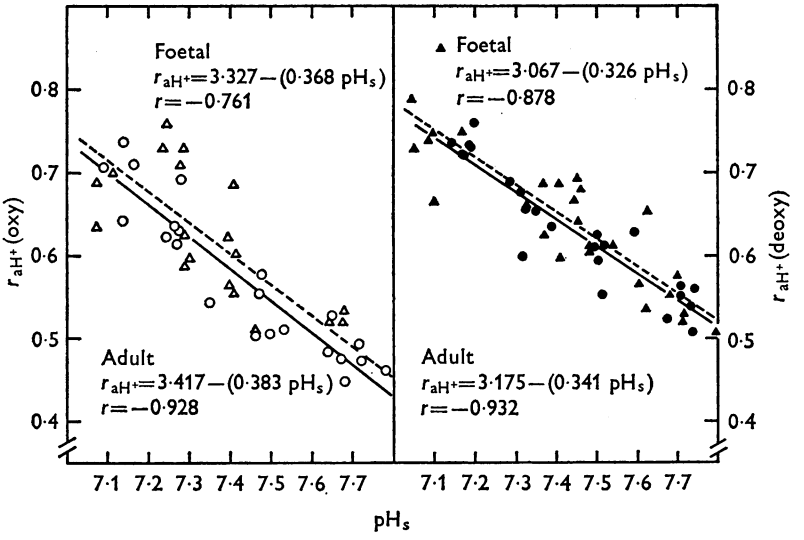

Fig. 2. Plot of the ratio of the hydrogen-ion activities of serum and red cells (r_{aH+}) as a function of serum pH (pH_s). The left panel shows the values obtained in oxygenated adult (○) and foetal (△) blood, the right panel the ones obtained in deoxygenated samples of adult (●) and foetal (▲) blood. The linear regression equations are likewise included.

TABLE 1. Calculated values of pK'_1 for serum and erythrocytes of human adult and foetal blood (mean ± S.D.)

	Adult		Foetal	
	Oxygenated	Deoxygenated	Oxygenated	Deoxygenated
Serum	6·11 ± 0·02	6·11 ± 0·01	6·10 ± 0·02	6·10 ± 0·02
Erythrocytes	6·10 ± 0·02	6·12 ± 0·02	6·10 ± 0·02	6·11 ± 0·03

DISCUSSION

The method according to which we have estimated the carbamate concentration in red blood cells includes the following assumptions which require a critical comment.

(1) The distribution of chloride and bicarbonate ions between the inside and the outside of the red cell is governed by the Donnan theory of membrane equilibria (Donnan, 1911). The fact that the distribution of chloride ions conforms to Donnan's theory was first shown to be true by van Slyke, Wu & McLean in 1923. The validity of their calculations has been verified by the work of other investigators (van Slyke, Hastings, Murray & Sendroy, 1925; Dill, Edwards & Consolazio, 1937; Fitzsimons & Sendroy, 1961; Funder & Wieth, 1966; Jay & Burton, 1969). A straightforward analysis of the bicarbonate ion distribution is complicated by the fact that carbon dioxide inside the red cell is bound in a form other than bicarbonate, namely carbamate. The careful study of Fitzsimons & Sendroy (1961) showed, however, that the distribution pattern of bicarbonate ions is similar to the one of chloride ions when allowance was made for the carbamate concentration with the data available at the time. It should be pointed out in this connexion that the calculation of the bicarbonate concentration in the red cell from r_{Cl^-} is not affected by the binding of either ion to plasma proteins or haemoglobin as long as it can be assumed that both ions are bound to the same extent. In any event, the binding of bicarbonate ions to haemoglobin appears to be so small (Roughton, 1970; Kilmartin & Rossi-Bernardi, 1971) that it can be ignored for our purposes.

(2) Other forms of bound carbon dioxide, e.g. carbamate bound to plasma proteins and carbonate (CO_3^{2-}) were not taken into account. The concentration of carbamate in horse serum is 0·5 mM at P_{CO_2} = 40 mm Hg, pH 7·4 and 38 °C (Stadie & O'Brien, 1937) which is less than 2 % of the bicarbonate concentration. Roughton (1935) on the other hand concluded from experiments in which the rapid uptake of carbon dioxide by serum was measured, that the amount of carbamino bound carbon dioxide in serum is negligible under physiological conditions. In view of these uncertainties a correction for carbamate bound to serum proteins was not applied. The concentration of CO_3^{2-} as the other possible form of bound carbon dioxide is negligible at physiological pH values. This is due to the low value of K_2', the apparent second dissociation constant of carbonic acid (Hastings & Sendroy, 1925).

(3) The activity coefficients of bicarbonate and chloride ions were considered to be equal in serum or red cell fluid. It is well known that according to the Debye-Hückel theory of interionic forces, ion activities rather than ion concentrations should be considered at physiological values of ionic strength. Accordingly, the relationship between the distribution of chloride and bicarbonate ions should be rewritten as follows:

$$r = \frac{\gamma_{Cl^-,c}[Cl^-]_c}{\gamma_{Cl^-,s}[Cl^-]_s} = \frac{\gamma_{HCO_3^-,c}[HCO^-_3]_c}{\gamma_{HCO_3^-,s}[HCO^-_3]_s}, \qquad (7)$$

whereby $\gamma_{c,\,s}$ denotes the activity coefficient of chloride and bicarbonate ions in red cells and serum respectively and the terms in brackets the molal concentrations of the ions.

To allow for both possible differences of ionic strength in serum and red cells and of the radius of chloride and bicarbonate ions we used the following form of the extended Debye-Hückel equation to estimate the pertinent activity coefficients

$$-\log \gamma = \frac{A_z + z - \sqrt{\mu}}{1 + d \times B \sqrt{\mu}}. \tag{8}$$

A and B are constants with numerical values of 0·512 and $3{\cdot}3 \times 10^7$, respectively; d is the effective diameter of the ion, z the charge of the ion and μ the ionic strength. For the chloride ions a Goldschmidt radius of 1·8 Å has been taken and for the bicarbonate ions the one which can be calculated from X-ray diffraction data of $KHCO_3$ crystals (Nitta, Tomiie & Koo, 1952) which is about 2·8 Å. These radii are probably larger in watery solutions due to the hydration of the ion but since the available data would not increase the conclusiveness of the calculation this effect was not taken into account. The value of μ was assumed to be 0·15 in serum and 0·17 in the red cell fluid. Inserting the calculated activity coefficients in eq. (7) and solving for $[HCO_3^-]_c$ shows that the intraerythrocytic bicarbonate concentration is only 0·2 % lower when calculated from ion activities rather than from ion concentrations. In view of these considerations it appears that neither of the assumptions we have made offers a serious theoretical obstacle to the method with which we estimated the carbamate concentration in erythrocytes.

The fraction of 'oxylabile carbamate' in erythrocytes. At a given P_{CO_2} and pH deoxyhaemoglobin binds more carbon dioxide in the form of carbamate than oxyhaemoglobin does, the difference being due to a fraction of carbamate which is linked to oxygenation. This was unequivocally shown to be true by Rossi-Bernardi & Roughton (1967) who furthermore gave ample theoretical evidence that the formation of carbamate occurs at the N-terminal amino groups of both α- and β- chains which was later experimentally verified by Kilmartin & Rossi-Bernardi (1969, 1971). From studies in haemoglobin solutions to which organic phosphates have been added it was inferred that the actual difference between the amount of carbon dioxide bound to oxygenated and deoxygenated haemoglobin ($\Delta HbCO_2/\Delta HbO_2$) is less than that assumed by Rossi-Bernardi & Roughton (1967), most likely due to an interaction of these substances and carbon dioxide at the terminal amino groups of the β-chains (Bauer, 1969, 1970; Pace *et al.* 1970; Siggaard-Andersen, 1971; Tomita & Riggs, 1971). The value of $-\Delta HbCO_2/\Delta HbO_2 = 0{\cdot}081$ at 37°C, $pCO_2 = 40$ mm Hg and

a red cell pH of 7·2 obtained in the present study (Fig. 1) is only about 30 % of the earlier figure published by Rossi-Bernardi & Roughton (1967). It is in good agreement, however, with the figure of 0·084 calculated by Rossi-Bernardi & Roughton (1970) in analysing the specific effect of carbon dioxide on the oxyhaemoglobin dissociation curve of human blood at pH_s 7·4. When comparison is made at pH_s 7·4 rather than at pH_c 7·2, $-\Delta HbCO_2/\Delta HbO_2$ comes out to be 0·092. This figure is somewhat higher than the one obtained at constant pH_c because of the additional effect of the pH shift accompanying oxygenation.

It should be noted at this point that the method we have applied to estimate red cell carbamate tends to overestimate this compound because of the necessarily long equilibration period of the blood samples which causes 2,3-DPG to fall. The maximal decrease of 2,3-DPG which was observed under the prevailing experimental conditions amounted to about 20 % at acid pH. According to Klocke (1972) this decrease would increase the fraction of 'oxylabile carbamate' by about 0·02 unit at pH_s 7·4. Since the fall in 2,3-DPG concentration is less at pH 7·4, one would expect the consecutive increase in 'oxylabile carbamate' to be in the order of 0·005–0·01 unit, a figure which hardly exceeds experimental error. Furthermore, it is reassuring to find that the fraction of 'oxylabile carbamate' obtained by Klocke (1972) with a rapid reaction apparatus is in excellent agreement with the present results for adult blood when related to serum rather than to red cell pH.

Under comparable conditions (pH 7·2, P_{CO_2} = 40 mm Hg, 37°C) foetal red cells give a value of 0·117 for $-\Delta HbCO_2/\Delta HbO_2$ which is significantly higher than the corresponding figure for adult red cells. This higher fraction of 'oxylabile carbamate' in foetal erythrocytes at constant pH_c and P_{CO_2} could be explained by a reduced affinity of 2,3-DPG to foetal haemoglobin (de Verdier & Garby, 1969) and/or by an intrinsically higher carbon dioxide affinity of foetal in comparison to adult haemoglobin. Further studies in purified solutions of foetal haemoglobin should give the correct answer.

Consistent with the amount of 'oxylabile carbamate' being higher in foetal blood is the finding that at pH_s 7·4 and a P_{CO_2} of 40 mm Hg, the pH difference between oxygenated and deoxygenated cells is smaller in foetal than in adult blood. This corresponds nicely to the smaller Bohr effect of foetal human blood when expressed at $\Delta \log P_{50}/\Delta pH$ which has been described by Hilpert, Fleischmann, Kempe & Bartels (1963). Since the alkaline Bohr effect of foetal and adult haemoglobin is identical in the absence of carbon dioxide (Antonini, Wyman, Brunori, Fronticelli, Bucci, Reichlin & Rossi-Fanelli, 1964) the lesser Bohr effect in foetal erythrocytes in the presence of carbon dioxide is likely to be due

to a more pronounced formation of 'oxylabile carbamate' in these cells which in the process of oxygenation would take up a more important part of the hydrogen ions coming from the oxylabile acid groups, thereby decreasing the Bohr effect (Rossi-Bernardi & Roughton, 1967). In addition, the direct influence of 2,3-DPG on the Bohr effect (Benesch, Benesch & Yu, 1969; Riggs, 1971; de Bruin, Janssen & van Os, 1971) would have to be considered. From the present data it is, however, not possible to draw any conclusion in this respect.

Estimation of the apparent carbamate equilibrium constants. The fraction of amino groups which at a given p_{CO_2} and pH have reacted with carbon dioxide to form carbamate can be described by the following equation (Stadie & O'Brien, 1937; Rossi-Bernardi & Roughton, 1967; Forster, Constantine, Craw, Rotman & Klocke, 1968)

$$f = \frac{K'_z . K'_c [CO_2]}{K'_z . K'_c [CO_2] + K'_z [H^+] + [H^+]^2}. \quad (9)$$

$[CO_2]$ and $[H^+]$ are the molar concentration of dissolved gas and hydrogen-ion activities respectively. K'_z is the apparent equilibrium constant for the ionization of the amino groups and K'_c the apparent equilibrium constant for the reaction of carbon dioxide with the uncharged amino groups to form carbamate. In deriving eq. (9) it is implicitly assumed that all amino groups involved in the binding of carbon dioxide are alike and behave independently, e.g. that the N-terminal α-amino groups of both α- and β-chains react with carbon dioxide to the same extent. This seems likely to be untrue in the presence of 2,3-DPG, the binding of which involves the α-amino groups of the N-terminal valines of the β-chains (Bunn & Briehl, 1970; Benesch, Benesch, Bank, Renthal & Bray, 1971). This binding shifts the ionization equilibria of these groups towards the protonated form (Riggs, 1971) and reduces therefore the carbon dioxide affinity of these particular groups.

In spite of the theoretical difficulties we considered it useful to derive these apparent constants after transforming eq. (9) into another form (Rossi-Bernardi & Roughton, 1967) namely,

$$\frac{[CO_2]}{[H^+]} \times \frac{1-f}{f} = \frac{1}{K'_c} + \frac{[H^+]}{K'_c . K'_z}, \quad (10)$$

in which f is the number of moles carbamate formed per mole of haemoglobin monomer under the prevailing experimental conditions (Fig. 1). A plot of the left-hand term against $[H^+]$ should give a straight line from which K'_c is obtained as the reciprocal of the ordinate intercept. K'_z can then be calculated from the slope of the line and K'_c. Fig. 3 shows a plot of this kind, whereby the two lines for the deoxygenated samples of adult and foetal blood proved to be significantly different. The numerical

values for K'_z and K'_c are listed in Table 2. It should be borne in mind that while K'_z and K'_c are useful if one wishes to calculate the carbamate concentration in human red cells, they do not have a solid theoretical basis. This is shown by the fact that both the contribution of the α-amino groups of the N-termini of the α-chains to the alkaline Bohr effect (Perutz, Muirhead, Mazzarella, Crowther, Greer & Kilmartin, 1969; Kilmartin &

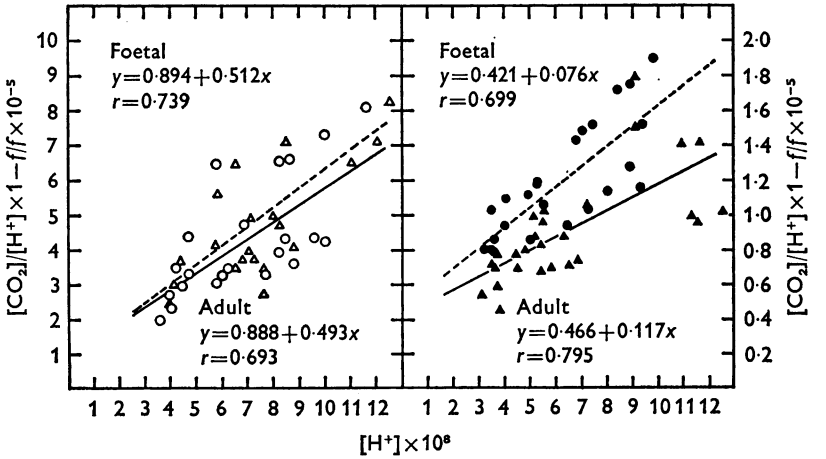

Fig. 3. Plot of the left-hand function of eq. (10) against the hydrogen-ion activity for oxygenated (left panel) and deoxygenated (right panel) adult (○, ●) and foetal (△, ▲) red cells. The linear regression equations from which K'_z and K'_c were calculated are likewise included.

TABLE 2. Calculated values of K'_z and K'_c for oxygenated and deoxygenated foetal and adult red blood cells

	Adult		Foetal	
	Oxygenated	Deoxygenated	Oxygenated	Deoxygenated
K'_z	$1{\cdot}79 \times 10^{-8}$	$3{\cdot}98 \times 10^{-8}$	$1{\cdot}74 \times 10^{-8}$	$5{\cdot}53 \times 10^{-8}$
K'_c	$1{\cdot}13 \times 10^{-5}$	$2{\cdot}15 \times 10^{-5}$	$1{\cdot}12 \times 10^{-5}$	$2{\cdot}38 \times 10^{-5}$

Rossi-Bernardi, 1969) and the displacement of 2,3-DPG from the N-terminal α-amino groups of the β-chains upon oxygenation of haemoglobin inevitably require an increase of K'_z whilst the treatment of our data leads to a decrease of K'_z. It would be appropriate therefore to call K'_c and K'_z obtained in this particular way 'apparent carbamate equilibrium constants', i.e. parameters governing the carbamate formation for a given set of conditions only.

The value of pK'_1 in erythrocytes. The absolute values of pK'_1 obtained in this study check well with the ones calculated by van Slyke *et al.*

(1923, 1925) for oxygenated red blood cells and also with the one obtained by Fitzsimons and Sendroy (1961). They are, however, consistently higher than the values which were obtained under the assumption that bicarbonate is the only form of bound carbon dioxide present in the red cell particularly in deoxygenated samples (van Slyke *et al.* 1925; Dill *et al.* 1937). Deane & Smith (1957), on the other hand, most likely over-estimated pK'_1 in red cells because they used for their calculations the figure for carbamate which was found in purified haemoglobin solutions. We found pK'_1 to be significantly lower in oxygenated adult red cells in comparison to deoxygenated ones which is most likely due to a higher ionic strength in oxygenated cells. An increase in ionic strength could be caused by the liberation of organic phosphates from haemoglobin upon oxygenation.

TABLE 3. Standard blood gas data in the arterial and mixed venous blood of resting man

	Arterial blood	Mixed venous blood
Oxygen saturation	98 %	75 %
P_{CO_2}	40 mm Hg	47 mm Hg
CO_2 concentration	21·4 mM	23·4 mM
pH_s	7·410	7·380
pH_c	7·190	7·170
Intracellular haemoglobin concentration (in monomers)	20 mM	
Haematocrit (adult)	0·45	
Haematocrit (foetal)	0·55	

The physiological role of carbamate in the respiratory cycle can be assessed on the basis of the data presented in Table 2 and Fig. 1. The standard values for arterial and venous blood of the resting man which were used in the pertinent calculations are listed in Table 3.

The calculation of the carbamate concentration in the red cells of arterial and mixed venous blood was done with the aid of eq. (9) using the values for K'_z and K'_c given in Table 2 and the value for the intracellular haemoglobin concentration given in Table 3. The concentration of dissolved carbon dioxide was obtained from the solubility coefficients given above and $[H^+]$ from the pH_c values listed in Table 3. Allowing for a haematocrit of 0.45, the carbamate concentration in arterial blood is then 0·36 mM and in the mixed venous blood 0·57 mM. These estimates were done on the basis of Ferguson's (1936) observation that the carbamate concentration is linearly related to the oxygen saturation of haemoglobin. The role of carbamate in carbon dioxide transport is then given by the fraction of the differences between carbamate concentration and total carbon dioxide concentration in arterial and mixed venous blood and amounts to 0·21/2·0

$\times 100 = 10{\cdot}5\,\%$. This figure is about 40 % of the one previously calculated (Rossi-Bernardi & Roughton, 1967). Similar calculations for foetal blood gave a carbamate concentration in the arterial blood of 0·40 mM and in the mixed venous blood of 0·78 mM. These figures were obtained with the same blood gas data listed in Table 3 with the exception that allowance was made for the higher haematocrit of foetal blood. Correspondingly, the role of carbamate in the carbon dioxide transport of foetal blood is $0{\cdot}38/2{\cdot}0 \times 100 = 19\,\%$.

The contribution of carbamate to the carbon dioxide exchange across the placenta during the last stages of gestation can also be assessed using eq. (9) and the values for K'_z and K'_c given in Table 2. The necessary blood gas data on blood from the umbilical artery and umbilical vein were taken from the review paper of Bartels & Wulf (1965). The carbamate fraction of the total amount of carbon dioxide which is transferred from the foetal blood to the maternal is just about the same as calculated for the example given in Table 3, namely 20 %. The degree of uncertainty in doing these calculations is, however, rather high because of the substantial degree of scattering in the blood gas data reported for human umbilical blood.

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