CARBAMINO COMPOUNDS OF CO₂ WITH HUMAN HÆMOGLOBIN AND THEIR ROLE IN THE TRANSPORT OF CO₂

By J. K. W. FERGUSON

(*From the Department of Physiology, Faculty of Medicine, University of Western Ontario, London, Canada*)

INTRODUCTION

A CHEMICAL method for the estimation of CO_2 bound to hæmoglobin by a carbamino linkage, i.e. bound to an —NH_2 group on the hæmoglobin molecule, was described by Ferguson & Roughton [1934a]. In their succeeding paper [1934b] evidence was presented that the carbamino compound must play a significant part in the transport of CO_2 by the blood. Only ox hæmoglobin was used in these experiments as difficulty was anticipated in applying the method to the hæmoglobin of most other animals. A tentative calculation, however, of the role of carbamino compound in the transport of CO_2 by human blood was made by combining the data obtained on ox hæmoglobin with the data of L. J. Henderson and his associates on the conditions of CO_2 transport in human blood.

It was obviously desirable to investigate directly, as soon as possible, the capacity of human hæmoglobin to form carbamino compounds. Furthermore, certain points connected with the method had, for lack of time, received rather summary investigation and obviously deserved further attention.

The work to be reported in this paper was undertaken with these two considerations in view and its scope may be outlined as follows:

(1) The chemical method of estimating carbamino CO_2 has been modified for use with solutions of human hæmoglobin.

(2) A more ambitious attempt has been made to estimate the loss of carbamino CO_2 during a determination. As a result it has become evident that the previous allowance for such loss was considerably too low.

(3) The utility of the chemical method has been further extended by the demonstration that it is applicable to solutions with low total CO_2 content. (The validity of the method under such conditions was previously under some suspicion.)

(4) The effect of varying pressures of CO_2 on the carbamino CO_2 content of solutions of human hæmoglobin, with base added to simulate conditions within the red blood corpuscle, has been investigated at CO_2 pressures ranging from 4 to 80 mm. Hg, and with the hæmoglobin fully oxygenated and fully reduced.

(5) To obtain data under conditions more nearly simulating those in the circulating blood, the effects of varying degrees of partial oxygenation of the hæmoglobin on the formation of carbamino compound have been determined.

(6) Finally, a simplified method, applicable under limited conditions, is proposed for the estimation of the importance of carbamino compound in the transport of CO_2.

NOMENCLATURE

The combination of CO_2 with an —NH_2 group on the hæmoglobin molecule may be represented by the equation

$$CO_2 + HbNH_2 \rightarrow HbNHCOOH.$$
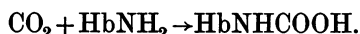
Hæmoglobo-carbamic acid was suggested by Ferguson & Roughton as a name for the carbamic acid so formed.

At hydrogen-ion concentrations which obtain in the red blood corpuscle, this acid would be largely dissociated to form $HbNHCOO^-$ [Stadie & O'Brien, 1935], and in this state would be referred to as hæmoglobo-carbamate. These names are particularly useful in discussion which requires that the ionized and un-ionized states be distinguished. For many purposes, however, it would be more convenient to use a shorter name to designate the total carbamino compound, i.e. ionized plus un-ionized. The name carbhæmoglobin [Henriques, 1928] would be eminently suitable and has been used in this sense by Groscurth & Haveman [1935]. It must be pointed out, however, that the indirect method of estimation employed by these authors cannot be regarded as measuring only carbamino-bound CO_2, but must include CO_2 or $\overline{H}CO_3$ bound to Hb by other linkages.

In this paper, carbhæmoglobin, represented by the symbol $HbCO_2$, will be used to mean total carbamino-bound CO_2 as measured directly by the chemical method.

SECTION I. EXPERIMENTAL METHODS AND CONTROLS

The hæmoglobin solutions were prepared by the method of Adair [1934]. The stroma was separated by the use of purified ether and salt. The excess of salt and ether was removed by dialysis. It was found that dialysis against 0·5 p.c. KCl instead of distilled water helped to keep the loss of oxygen capacity of these solutions at a minimum during the time which must elapse before they could be used up. The solutions were kept frozen at about $-3°$ C. in an electric refrigerator till they were used. In the week which usually elapsed before one preparation could be used up the loss in O_2 capacity did not amount to more than about 5 p.c.

The method of analysis for $HbCO_2$ will be outlined briefly, with detail sufficient only to make clear the modifications which have been introduced.

Combined CO_2 other than $HbCO_2$ is precipitated as $BaCO_3$ by the addition of alkaline $BaCl_2$. The $BaCO_3$ is removed by centrifuging. The procedure is to mount three 5 c.c. syringes (one containing the solution to be analysed and the two others the reagents) in a water thermostat, with their plungers resting on a movable platform. When the platform is elevated the three syringes are emptied simultaneously and their contents are expelled through a Hartridge-Roughton rapid mixer. The mixed fluid is caught in a centrifuge cup surrounded by ice. The precipitate of $BaCO_3$ is removed by centrifuging at 2000 r.p.m. for 10 min. The supernatant liquid is then analysed for CO_2 by the van Slyke method. The result gives the uncorrected or gross $HbCO_2$, which may be expressed as c.c. of CO_2 per 100 c.c. of original hæmoglobin solution.

The main difficulty encountered in applying the method to solutions of human hæmoglobin was a great exaggeration of the difficulty previously encountered in solutions of ox hæmoglobin containing low concentrations of total CO_2; namely, the "protective action" of the protein which hindered the complete separation of the $BaCO_3$ precipitate by centrifuging. In the case of ox hæmoglobin the difficulty has been overcome by increasing the bulk of the precipitate by adding sufficient $NaHCO_3$ with the reagents to bring the total CO_2 content of the system to 50 mM. per litre of original hæmoglobin solution. With human hæmoglobin, eight or ten times as much carbonate had to be added and even this amount was not as effective in securing complete removal of the $BaCO_3$ as the smaller quantity had been in ox hæmoglobin.

The usual blank correction for $BaCO_3$ remaining in the supernatant fluid after centrifuging was, for human hæmoglobin, 1 c.c. of CO_2 per 100 c.c. original hæmoglobin solution as against 0·2–0·4 c.c. for ox hæmoglobin.

It might be conjectured that the great bulk of precipitate used for human hæmoglobin would drag down a considerable amount of adsorbed hæmoglobin on centrifuging. Actually any such tendency proved to be negligible, for colorimetric estimation of the hæmoglobin in the supernatant fluid showed it to be present in exactly one-third the concentration of the original solution.

One preparation of human hæmoglobin was found to retain its protective action in spite of high concentrations of added carbonate. It has not yet been determined whether this was a personal characteristic, or whether a variation in the method of preparation was responsible.

Reagents for human hæmoglobin.

Human hæmoglobin was found to stand alkali in amounts sufficient for the determination without detectable destruction. It was found satisfactory for the purposes of the analysis to add to the hæmoglobin solution an equal volume of NaOH solution of a strength (in mol./litre) given by the formula: $[NaOH] = 12 [Hb] + [CO_2]$.

$[Hb] = O_2$ capacity of the Hb solution in mol./litre. $[CO_2] =$ total CO_2 content of the Hb solution in mol./litre.

The carbonate for increasing the bulk of the precipitate was placed in the same syringe as the alkali. Since its concentration was large compared with variations in the CO_2 content of the hæmoglobin solution, it was always used in syringe C at a constant concentration of about 0·4 mol./litre.

TABLE I. Contents of syringes for the analysis of $HbCO_2$ in human hæmoglobin solutions

Syringe	Content
A	Hb solution to be analysed.
B	$BaCl_2$ (0·6 mol./litre).
C	$NaOH + Na_2CO_3$ (for concentrations see text).

Corrections

The analysis of the supernatant fluid gives a figure for the gross $HbCO_2$ (expressed as c.c. of CO_2 per 100 c.c. of original Hb solution), and to this figure the following corrections must be applied to obtain the preformed $HbCO_2$ in the original hæmoglobin solution. To correct:

(1) For dissolved CO_2 which turns into $HbCO_2$ on the addition of alkali, subtract 10 p.c. of the dissolved CO_2 in the original hæmoglobin solution.

(2) For incomplete removal of $BaCO_3$ by centrifuging, subtract 1 c.c. of CO_2 per 100 c.c. original hæmoglobin solution.

(3) For loss of $HbCO_2$ by dissociation during the determination, multiply the figure remaining after the two previous corrections have been deducted by 1·5.

The first two corrections are adequately discussed in the previous publication [Ferguson & Roughton, 1934a]; the last is new and requires attention in detail.

The loss of $HbCO_2$ during an estimation

Ferguson & Roughton found that prolonging the centrifuging for a second period of fifteen minutes resulted in a loss of only about 8 p.c. of the $HbCO_2$. This figure was used as a minimum correction for loss of $HbCO_2$ during the estimation. It was realized that the loss might be considerably greater, particularly in the interval after mixing, when (1) the mixture was not yet chilled sufficiently to stabilize the $HbCO_2$ and when (2) the bulk of the precipitate was not yet removed by centrifuging.

It seemed desirable to investigate the rate of dissociation of $HbCO_2$ in the presence of the alkaline $BaCO_3$ at different temperatures in the hope of making a more precise estimate of the loss of $HbCO_2$ which might occur during a determination.

The hæmoglobin solution was mixed with an equal volume of sodium carbonate solution ($0.4M$) with NaOH of the strength used in an ordinary determination. The flask was kept for twenty minutes in a water bath at the required temperature. Under these conditions (pH $ca.$ 11–12, and high carbonate concentration) considerable amounts of $HbCO_2$ were formed. $BaCl_2$ solution was now added from another flask which had been standing in the same bath. The mixture was poured into six test-tubes, also standing in the bath. One tube was then centrifuged immediately (at a temperature as close as possible to that of the bath) and the remainder at appropriate intervals thereafter. Immediately before it was to be centrifuged each tube was inverted ten times to stir up the heavy precipitate.

On the addition of the $BaCl_2$ the concentration of dissolved carbonate and, a $fortiori$, the pressure of CO_2 in the mixture would become vanishingly small. Accordingly the CO_2 combined to hæmoglobin would, as it dissociated off, be removed immediately by hydration (carbonic anhydrase being active in the solution), and precipitated as carbonate. As each tube was centrifuged in turn, the $HbCO_2$ remaining in the supernatant fluid was found to be progressively less. Thus a curve could be plotted

showing the rate of dissociation of $HbCO_2$ at that particular temperature and in the mixture used in an ordinary analysis. Fig. 1 shows three such curves at the temperatures 1·5, 18 and 37° C. respectively.
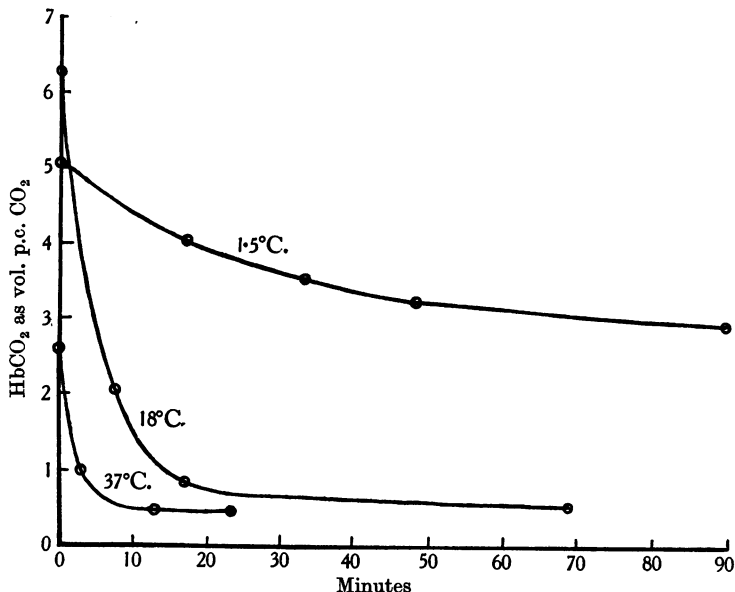

Fig. 1. Rate of dissociation of $HbCO_2$ in alkaline $BaCl_2$ at different temperatures (ox Hb).

These curves show a number of points of interest. Firstly, so far as they have been investigated they can be fitted by equations of the mono-molecular type. Consequently the rate of dissociation at any time can be represented by a differential equation of the type

$$\text{Rate of reaction} = \frac{d\,[\text{HbCO}_2]}{dt} = k\,[\text{HbCO}_2],$$

$t =$ time in seconds.

Secondly, the variation of k (the velocity constant) with temperature does not quite obey the Arrhenius equation. That is:

$$\ln k \neq \frac{C}{T},$$

$T =$ absolute temperature.

This is not strange because the molecular species dissociating is, according to theory, HbNHCOOH (hæmoglobo-carbamic acid), and not $HbCO_2$ which is the entity measured by the analysis. The concentration of HbNHCOOH would not likely be the same fraction of the total carb-hæmoglobin at different temperatures.

Thirdly, the rate of dissociation, particularly at the higher temperatures, proved to be greater than had been expected. For example at 37° C. the time of half completion was about 3 min. This velocity would allow considerable loss of $HbCO_2$ were it not checked very soon after mixing.

In an analysis the reagents were mixed at 37·5° C. and expelled into a centrifuge cup surrounded by ice where the mixture cooled down fairly rapidly. The actual rate of cooling could be followed easily enough by means of a thermometer in the cup. From a curve of cooling thus obtained, the temperature at any time after mixing could be read off. By plotting the logarithms of the velocity constants obtained from Fig. 1 against the reciprocal of their corresponding temperatures in absolute units, a velocity constant k could be interpolated with sufficient accuracy for any temperature between 37 and 0° C. Now a curve could be plotted between k and t, where k is the velocity constant and t is the time (in seconds) after mixing. The area under this curve could be computed over any finite range and thus a value for the corresponding finite integral obtained.

Now if $A = [HbCO_2]$ at time of mixing and if $A - X = [HbCO_2]$ at time t after mixing, it can be shown that

$$ln \frac{A}{A-X} = \int_{t=0}^{t=t'} kds.$$

That is to say the computation of the area under the curve of k plotted against t enables the calculation of the loss of $HbCO_2$ during the interval from $t=0$ to $t=t'$.

The time t' is the time required to balance the centrifuge cups, to set the centrifuge in motion, and to separate the bulk of the precipitate. It was not very easy to evaluate this time precisely but 90 sec. may be considered a fair average value for t'. (Loss during the remaining 9 min. of centrifuging has been neglected. 90 p.c. or more of any carbonate formed by the dissociation of carbhæmoglobin after the separation of the bulk of the precipitate would remain in the supernatant fluid adsorbed to the protein. The further centrifuging serves only to pack down the precipitate.)

When $t' = 90$ sec. the graphical integration of the curve gives a value of ln 1·5 for the expression $ln \frac{A}{A-X}$. Therefore $A = 1·5 (A - X)$. In other words the approximate correction to be applied for loss during a determination is 1·5.

The curves shown in Fig. 1 are for ox hæmoglobin. It was not practicable to obtain satisfactory curves on human hæmoglobin at 37° C. because of the rapidity with which it is denatured by the alkali at that

temperature. At lower temperatures, however, the velocity constants were found to be of about the same magnitude for human as for ox hæmoglobin when the strength of alkali added to each was that used in ordinary determinations on each.

Loss of $HbCO_2$ in previously published experiments

In the experiments of Ferguson & Roughton on ox hæmoglobin, an angle centrifuge was used and, consequently, accurate balancing of the centrifuge cups was not necessary. Accordingly the time t' must have been considerably shorter than 90 sec. An average value for t' in those earlier experiments might have been about 25–30 sec. With such a value for t' the correction factor would be about 1·2.

Means of increasing the precision of the method

The foregoing experiments emphasize the importance of knowing as accurately as possible the time (t') elapsing between the mixing and the separation of the bulk of the precipitate. If a large angle centrifuge is available and handily placed, the separation time can be shortened to about 15 sec. With this value for t' the factor should only be about 1·1. It could be reduced still further by the adoption of a more rapid method of cooling the mixture.

Solutions with low total CO_2 content

On the basis of some rather complex control experiments which gave ambiguous results with solutions of low total CO_2 content Ferguson & Roughton did not feel justified in claiming that the method was applicable to such solutions. The question was whether the extra carbonate added in the rapid mixer was effective in causing the centrifuging down of carbonate which had been previously molecularly dispersed in the hæmoglobin solution, particularly if this previously dispersed carbonate were small in amount, in which case it might be the more completely surrounded by adsorbed protein. The problem was to obtain a low concentration of carbonate molecularly dispersed in the hæmoglobin solution but unaccompanied by any $HbCO_2$. The clue to obtaining the latter condition is given in Fig. 1. In the presence of alkaline $BaCl_2$ at 37° C. the $HbCO_2$ would almost entirely dissociate in a few minutes leaving only carbonate molecularly dispersed in the hæmoglobin solution. The effectiveness of carbonate added in the rapid mixer would be de-

monstrated if, after centrifuging, the supernatant fluid contained only minimal amounts of CO_2.

Experimental. Hæmoglobin solutions containing 2–10 vol. p.c. of total CO_2 were mixed with alkaline $BaCl_2$ and kept at 37° C. for 30 min., in which time practically all of the $HbCO_2$ would have dissociated. To the mixture was now added (in the rapid mixer) a solution of Na_2CO_3 of the strength used in an ordinary analysis. The precipitate of $BaCO_3$ was then immediately removed by centrifuging as in an ordinary analysis.

On analysing the supernatant fluid for CO_2, a typical experiment gave a value of 0·4 c.c. CO_2 per 100 c.c. original hæmoglobin solution. When water was added in the rapid mixer, instead of Na_2CO_3, nearly all of the carbonate previously present remained in the supernatant fluid after centrifuging.

Apparently then, the method can be used with confidence for solutions containing low concentrations of total CO_2.

SECTION II. THE PHYSIOLOGICAL IMPORTANCE OF CARBHÆMOGLOBIN

Total CO_2 and $HbCO_2$ dissociation curves in solutions of human hæmoglobin

Experimental. The hæmoglobin solutions used had as a rule an oxygen capacity of 20–22 vol. p.c. By the addition of Na_2CO_3 they were adjusted so that at a pressure of CO_2 of 40 mm. Hg they would have a pH of 7·1–7·3. This range may be regarded as including the normal range of pH in red blood corpuscles under resting conditions, at that pressure of CO_2. The approximate pH of the solutions was calculated by the Henderson-Hasselbalch formula using 6·1 arbitrarily as the value of pK_1'.

For the determination of one set of curves about 50 c.c. of solution were adjusted to the appropriate content of base and were used up within three days. To prevent deterioration the solution was kept on ice all day and frozen each night.

One set of curves is intended to show the effect of varying CO_2 pressures on the $HbCO_2$ content and total CO_2 content of the solution with the hæmoglobin fully reduced and fully oxygenated. In each set of curves it was practicable to obtain points at four or five different pressures of CO_2 and the range covered was from 4 to 80 mm. Hg. About 7 c.c. of solution were placed in a tonometer immersed in a water thermostat at a temperature of 37·5° C. and there equilibrated with a mixture of CO_2 and air (for oxygenated solutions) or with CO_2 and N_2 for reduced solutions.

The gas and liquid phases were separated by the method of Austin *et al.* [1922]. About 5 c.c. of the solution were drawn into syringe A to be analysed for $HbCO_2$; the remainder was analysed for total CO_2 and O_2 in the van Slyke apparatus. A sample of the gas phase was analysed for CO_2 in the Haldane apparatus.

Results. Fig. 2 shows one set of curves; total CO_2 and $HbCO_2$ dissociation curves on oxygenated and reduced hæmoglobin. The data for two other full sets and a number of partial sets at different values of pH support the same general conclusions and are given in Table II.
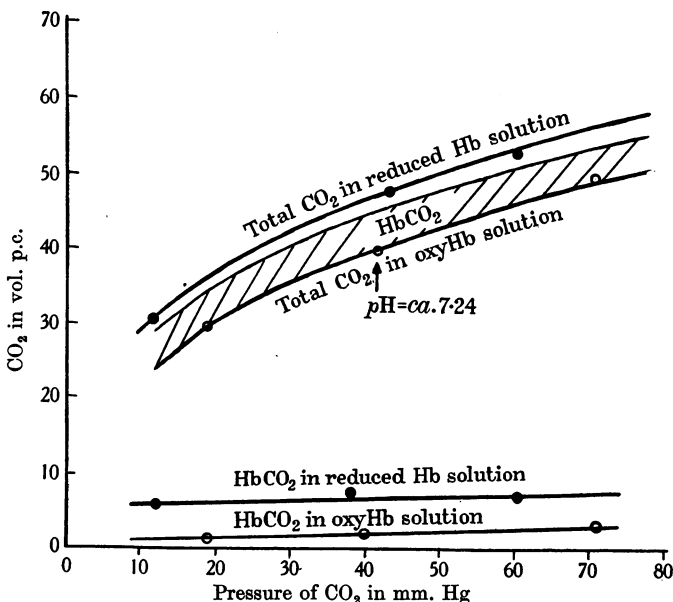

Fig. 2. Change in total CO_2 and $HbCO_2$ with changing P_{CO_2} in a solution of human Hb.

As in the previous work on ox hæmoglobin it was found that at the same pressure of CO_2 the reduced hæmoglobin solution invariably contained more $HbCO_2$ than did the oxygenated solution. The shaded area between the total CO_2 dissociation curves of oxy- and reduced hæmoglobin represents the proportion of the extra total CO_2 capacity of reduced hæmoglobin, which can be accounted for by the greater carbhæmoglobin content of the reduced hæmoglobin solution. It can be seen from Fig. 2 that 70–75 p.c. of the extra total CO_2 capacity (Haldane effect) can be so accounted for.

A very important characteristic of the carbhæmoglobin dissociation curves is that they are practically flat over a considerable range of CO_2

TABLE II

Date	Total CO₂ in vol. p.c.	Pressure of CO₂ mm. Hg	O₂Hb as vol. p.c. O₂	Corrected HbCO₂ vol. p.c.	Calcu- lated pH	HbCO₂* / O₂Hb
26. v. 35	37·4	51·0	19·1	0·2	7·12	−0·25
	45·7	50·0	0·6	4·8	7·17	
24. viii. 35	49·5	31·5	1·30	6·16	7·41	−0·24
	54·4	41·8	1·41	5·70	7·33	
	34·8	13·9	1·33	4·50	7·62	
	58·4	58·5	1·27	7·20	7·20	
	42·8	30·3	19·20	1·77	7·41	
	53·6	60·3	19·00	2·10	7·19	
23. x. 35	15·9	4·45	1·57	2·31	7·78	−0·16
	32·2	19·60	0·80	4·45	7·43	
	58·7	80·50	1·08	4·48	7·08	
	45·7	43·00	1·20	5·05	7·24	
	15·6	8·28	20·60	0·90	7·54	
	27·9	21·30	20·80	1·86	7·36	
	48·7	74·20	19·00	2·86	7·04	
	39·0	45·30	20·30	1·92	7·17	
4. xii. 35	65·2	33·50	1·54	10·10	7·50	−0·33
	61·5	40·00	21·60	3·52	7·43	
5. xii. 35	43·1	59·70	21·24	3·00	7·07	−0·24
	49·0	38·40	1·18	7·80	7·30	
8. xii. 35	30·6	12·30	1·92	5·80	7·59	−0·27
	52·6	60·50	0·96	6·90	7·13	
	47·0	42·00	1·20		7·24	
		38·00		7·00		
	49·0	71·00	21·24	3·00	7·06	
	29·3	19·00	22·05	1·14	7·45	
	38·9	41·60	22·15	1·40	7·21	

* This ratio is calculated for the physiological range of CO₂ pressure, viz. 40–60 mm. Hg.

pressure. As previously pointed out, this is to be explained by the opposing effects exerted on the carbhæmoglobin equilibrium by a rise of CO₂ pressure *per se*, and the rise in acidity which accompanies it. In these solutions the opposing effects appear to cancel each other rather exactly over a range of CO₂ pressures from 15 to 80 mm. Hg. It seems then that over the physiological range of CO₂ pressure for resting conditions, the HbCO₂ dissociation curves may be regarded as practically flat. That is to say, in these solutions variations in HbCO₂ concentration are due almost entirely to changes in the degree of oxygenation of the hæmoglobin rather than to changes in CO₂ pressure.

Effect of partial oxygenation on the formation of HbCO₂

Claims for the physiological importance of carbhæmoglobin must be based largely on the effect of oxygenation on carbhæmoglobin formation. In calculating the probable role of HbCO₂ in CO₂ transport it has been

tacitly assumed that at intermediate stages of oxygenation of the hæmoglobin the concentration of $HbCO_2$ would be linearly proportional to the concentration of reduced hæmoglobin. So far, direct investigation has been made only on the effects of complete oxygenation and complete reduction. It seemed desirable to test the effect of intermediate degrees of oxygenation.

Experimental. About 50 c.c. of hæmoglobin solution with an appropriate amount of Na_2CO_3 added were divided into seven portions. Each was equilibrated at 37° C. with the same pressure of CO_2 (40 mm. Hg) but with different pressures of O_2. The solution was then analysed for total CO_2, O_2, and $HbCO_2$.
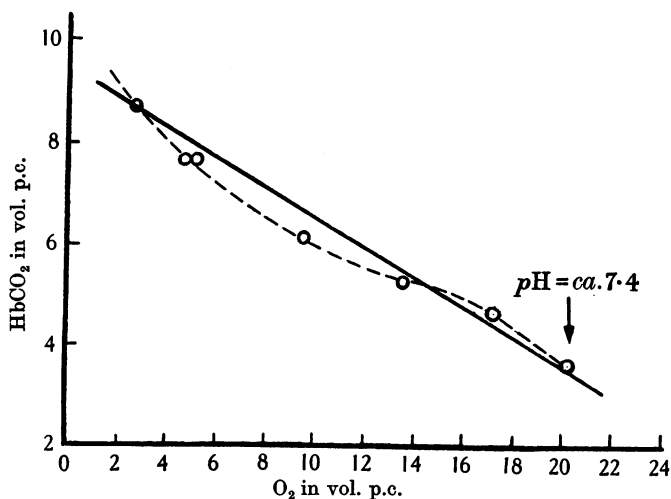

Fig. 3. Change in $HbCO_2$, with varying oxygenation of the Hb
at constant P_{CO_2} (human Hb).

Results. Fig. 3 shows the concentration of $HbCO_2$ plotted against the oxyhæmoglobin concentration. The data suggest a slightly inflected curve rather than a straight line, but the deviations from a straight line are not beyond experimental error. Apparently then, it is permissible to assume a linear relation between the $HbCO_2$ content and the degree of oxygenation of the hæmoglobin. It may be expressed thus:

$$[HbCO_2] = C - K\,[O_2Hb],$$

and therefore
$$\frac{d\,[HbCO_2]}{d\,[O_2Hb]} = -K.$$

To put it verbally, the absorption by the hæmoglobin of a given volume of oxygen results in the expulsion of a definite volume of CO_2 from combina-

tion as carbhæmoglobin; the amount being determined by the magnitude of K.

For the data of Fig. 3 with concentrations expressed as vol. p.c., $C = 9.6$ and $K = 0.3$. An average value for K, for human hæmoglobin, from experiments on six different samples of human hæmoglobin at pH's ranging from 7.2 to 7.4 (at 40 mm. Hg pressure of CO_2) is about 0.24. This constant may be used to calculate the role of $HbCO_2$ in the transport of CO_2 by human blood.

The role of carbhæmoglobin in CO_2 transport

Because of the flatness of the $HbCO_2$ dissociation curves in these solutions it is possible to calculate (knowing K) the change in $HbCO_2$ if only the change in O_2Hb is known. The flatness of the curve is due to the buffer power of the solution being such that the change in acidity associated with a given rise in CO_2 pressure is just sufficient to nullify the tendency of the rise in CO_2 pressure to cause a greater formation of $HbCO_2$. The hæmoglobin solutions in these experiments have approximately the same buffer power as normal whole human blood. It seems fair then to assume that in the red cell in normal blood the same flatness of the carbhæmoglobin dissociation curve would obtain, and hence that changes in $HbCO_2$ in the whole blood may be calculated from changes in the degree of oxygenation of the whole blood without special consideration of changes of CO_2 pressure, provided that the concentrations of other acids in the blood do not change appreciably.

Under resting conditions then, the role of carbhæmoglobin transport of CO_2 may be calculated from a knowledge of the respiratory quotient for any short period of time. Thus with an R.Q. of 0.8 the absorption of 10 c.c. of O_2 is accompanied by the output of 8 c.c. of CO_2. But the combination of 10 c.c. of O_2 with hæmoglobin will simultaneously expel 2.4 c.c. of CO_2 from combination as carbhæmoglobin. This will comprise

$$\frac{2.4}{8.0} \times 100 = 30 \text{ p.c. of the total } CO_2 \text{ evolved in the lungs.}$$

For the sake of precision it should be mentioned that not quite all of the 10 c.c. of O_2 absorbed in the lungs is combined with hæmoglobin, but that as much as 0.3 c.c. may remain in simple solution in the blood. This quantity, however, is scarcely significant in the present calculation.

Thus it seems probable that under resting conditions as much as 30 p.c. of the CO_2 transported by the blood, that is to say, evolved in the lungs, may be carried as carbhæmoglobin.

It is not yet possible to estimate with any confidence the importance

of carbhæmoglobin in conditions of exercise because of the complication introduced by the accumulation of lactic acid.

In the changes in CO_2 content of the red cells during the respiratory cycle carbhæmoglobin must play an even greater part than it does in the changes of the whole blood. Probably at least 70 p.c. of the transport of CO_2 by the red cells is accomplished by changes in the concentration of $HbCO_2$ in the cells.

Comparison of ox and human hæmoglobin

In solutions of reduced ox hæmoglobin at physiological pH and pressures of CO_2 of 40–60 mm. it was found that $HbCO_2$ comprised 8–10 p.c. of the total CO_2. With human hæmoglobin under the same conditions, $HbCO_2$ comprises 10–16 p.c. of the total CO_2. This greater apparent affinity of human hæmoglobin for CO_2 is due partly to the greater corrections for loss of $HbCO_2$ which have been used in calculating the results on human hæmoglobin. It was pointed out, however, in an earlier section of this paper that a comparable correction applied to the results previously reported for ox hæmoglobin would not increase the figures by more than 20 p.c. It seems evident then that many samples of human hæmoglobin really possessed a greater capacity than ox hæmoglobin to form carbhæmoglobin. The results on oxygenated hæmoglobin support the same conclusion. In oxygenated solutions of ox hæmoglobin $HbCO_2$ comprised 2–4 p.c. of the total CO_2. In oxygenated solutions of human hæmoglobin it comprises 2–7 p.c. These figures are, however, less reliable than those for reduced solutions because they are smaller and greatly affected by corrections.

The greater affinity of human hæmoglobin for CO_2 may be attributed to the greater buffer power of human hæmoglobin, as suggested to me in a personal communication by Dr F. J. W. Roughton. This greater buffer power implies:

(1) A greater number of free $—NH_2$ groups at a given pH, and hence more groups to react with CO_2;

(2) It also implies more base to neutralize hæmoglobo-carbamic acid as it is formed, with less change of pH;

and thus may act in one or both of these ways.

Carbhæmoglobin and former views of CO_2 transport

For many years now the greater CO_2 capacity of reduced blood has been ascribed to a difference in acid strength of the hæmoglobin in the reduced and oxygenated states. It might be timely to point out here that

the recent work on carbhæmoglobin in no sense refutes such a change in acid strength or its physiological importance. Although a large part of the extra combined CO_2 in the reduced hæmoglobin solution has now been shown to be combined directly to the hæmoglobin as $HbCO_2$ it must be borne in mind that this $HbCO_2$ is an acid (hæmoglobo-carbamic acid) which requires base to neutralize it no less than does carbonic acid. The base liberated when the hæmoglobin becomes a weaker acid on losing its oxygen enables hæmoglobo-carbamic acid to be neutralized without taking base from bicarbonate.

Not only does carbhæmoglobin fit into the classical picture of CO_2 transport with remarkably little disturbance to the classical design, but it has helped to explain some anomalies in the classical scheme. For example it has been known for some years [Roughton, 1935; Stadie & Hawes, 1928] that the apparent value for pK_1' of carbonic acid is different in the presence of reduced and oxygenated hæmoglobin. The anomalous change in pK_1' has been partly explained by the discovery of carbhæmoglobin.

SUMMARY

1. The chemical method of estimating carbamino compounds of CO_2 with hæmoglobin is shown to be applicable to solutions of human hæmoglobin when suitable measures are adopted to overcome the great "protective action" of this protein on the precipitate of $BaCO_3$.

2. From measurements on the rate of dissociation of $HbCO_2$ in alkaline solutions at different temperatures, the loss of $HbCO_2$ during determinations under various conditions is calculated. Implications regarding the precision of the method are discussed.

3. The chemical method is shown to be applicable to solutions of low total CO_2 content.

4. Carbamino compounds of CO_2 with human hæmoglobin and ox hæmoglobin have the same general properties. One of the most important of these from the physiological point of view is that oxygenation greatly diminishes the affinity of hæmoglobin for CO_2.

5. Higher figures are reported in this paper for the amounts of CO_2 combined with human hæmoglobin than were previously reported for ox hæmoglobin under comparable conditions. Reasons for this are discussed.

6. A higher estimate, too, is made of the physiological importance of $HbCO_2$ in the transport of CO_2. It is calculated that about 30 p.c. of the total CO_2 transport at rest, and about 75 p.c. of the transport in the red cells is by $HbCO_2$.

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