THE INFLUENCE OF CARBON DIOXIDE ON THE INTERCHANGE OF IONS BETWEEN THE CORPUSCLES AND THE SERUM OF BLOOD.

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CONTENTS.

Modern views regarding (*a*) the transport of carbon dioxide in the blood and (*b*) the maintenance of the neutrality of the blood, are based on Hamburger's hypothesis that the red cell is freely permeable to anions. The doctrine of the impermeability of the red cell to kations has been put forward by a large number of observers—Gürber(1), Höber(2), Koeppe(3), De Boer(4), Overton(5), Doisy and Eaton(6), and Mukai(7)—and, in fact, has met with general acceptance. According to Hamburger(8), however, the cell envelope is not impermeable to kations. He regards the sodium and potassium ions as existing in a state of dynamic equilibrium with one another, and able to interchange with one another under certain conditions. Thus, when the quantity of carbon dioxide in equilibrium with blood is increased from 10 p.c. to 20 p.c., potassium passes into the corpuscle and sodium out.

The first experimental observations on the effect of carbon dioxide on the blood were made by Zuntz in 1867. He found that the alkali of the serum was increased in amount when blood was submitted to high tensions of carbon dioxide. Zuntz assumed that this phenomenon was due to the carbon dioxide splitting sodium from a sodium protein compound. The liberated sodium combined with carbon dioxide and formed sodium carbonate. By further absorption of carbon dioxide sodium bicarbonate was produced, which, by a process of diffusion, became equal in amount on both sides of the cell.

The permeability of red cells to the chloride ion was demonstrated by Nasse (9) in 1874, and subsequently, Hamburger and his school showed the general permeability of the red cell to anions (—Cl, —PO_4, —SO_4, etc.). On Hamburger's hypothesis the Zuntz phenomenon, *i.e.* the increased quantity of alkali present in serum after the treatment of blood with carbon dioxide, is assumed to be due to the passage of anions—chloride mainly—from the serum into the cell and not to the passage of sodium from the cell into the serum.

Gürber's suggestion that carbonic acid reacts with the sodium chloride present in serum forming sodium bicarbonate and hydrochloric acid, of which the latter diffuses into the cell, is an expression of these observations. Such an action might take place to some extent if the active mass of carbonic acid were large compared to that of the sodium chloride. But the fact that considerable quantities of sodium chloride are present, not only in the serum but also in the red cells, and that these cells are freely permeable to carbon dioxide appears to form an effective barrier against the acceptance of Gürber's hypothesis.

Quantitative experiments by Van Slyke and Cullen (10) have shown that after treating blood with carbon dioxide at high tension, only about one half of the additional carbon dioxide carried by serum can be accounted for by the change in the chloride ion concentration. These observations accord with those of McLean, Murray and L. J. Henderson (11), who find that with varying tensions of carbon dioxide only two-thirds of the bicarbonate is accounted for by chloride. On the other hand Fridericia (12) and Mukai (7) both find that the increase in the carbon dioxide capacity of the serum is exactly equivalent to the chloride loss.

The observations on the distribution of chloride and bicarbonate ions between the red cells and the serum, and the impermeability of the red cells to kations, have been submitted to mathematical analysis by Warburg (13), and according to him may be explained on Donnan's theory of the distribution of permeating ions in two phase systems containing non-permeating ions.

In the following pages the effects of carbon dioxide on the distribution of electrolytes between the cells and serum of blood are recorded. The correlation of the ionic interchanges between the corpuscles and serum, and the variations in the carbon dioxide carrying powers of these fluids, do not support the hypothesis that the red cell is permeable to anions only. Kations move freely across the envelope of the red cell under the influence of carbon dioxide and take an important part in the maintenance of the neutrality of the blood, and the transport of carbon dioxide. Many of the experiments were carried out on blood saturated with carbon dioxide. It has been stated by Overton that results obtained after saturating the blood with carbon dioxide do not represent the changes which take place in the animal when the tensions of carbon dioxide vary between small limits only. This objection would be valid if (*a*) the red cell functions as a living cell, since high concentrations of carbon dioxide have a pronounced narcotic action on living tissues or (*b*) carbon dioxide decomposes substances in the red cell which are involved in preserving the neutrality of the blood. In point of fact the results observed on varying the carbon tension from 0 mm. to 40 mm. Hg, and from 0 mm. Hg to saturation vary in degree only. Further, the ionic interchanges which occur between the serum and corpuscles at high carbon dioxide tensions are completely reversible when the tension is lowered. Therefore the two objections are untenable and the results obtained on saturating blood with carbon dioxide may be accepted as showing in a gross degree the changes which occur with small variations in carbon dioxide tensions.

The experiments were made on fresh sheep's blood. It was impossible to carry out all the experiments on one sample of blood. Therefore, in order to correlate the results it was necessary to carry out the analyses on a series of different bloods and to average the figures. Each analysis is the result of two determinations which did not differ by more than 1 p.c.

The relative volumes of red cells and serum in blood.

Some of the results recorded depend upon the relative volumes of cells and serum in blood. To determine these values, 50 c.c. of the blood under examination was submitted to prolonged spinning in a powerful centrifuge (about ½ hour at 8000 to 10,000 revolutions per minute). At the end of this time the serum was carefully pipetted off, and measured. This method raises the question whether the degree of centrifugal force affects the relative volumes of cells and serum; that is, whether the fluid can be expressed from the red cells by centrifugal force. With any sample of blood a constant value can be obtained by prolonged spinning, beyond which no further separation can be made. This might be due to an equilibrium set up between the centrifugal force and the altered osmotic pressure of the red cell. To settle this question the volume of the red cells contained in a definite quantity of blood was determined. The red cells were then suspended in a volume of Ringer solution equal to that of the original blood and again centrifuged. The corpuscular volumes in each case were identical. The specific gravities of the serum and the Ringer solution differed widely—1032 and 1010 respectively—and therefore the centrifugal force to which the corpuscles were submitted differed to a corresponding degree. Hence it appears that on spinning blood the corpuscles are not compressed and that the values obtained by this method give the true corpuscular volume.

Many of the experiments are recorded under the terms: "Normal blood," "Carbon dioxide free blood," and "Carbon dioxide saturated blood." "Normal blood" implies blood which has been put into equilibrium with the alveolar air of the lungs. The term "CO_2 free blood" is applied to blood through which CO_2 free air has been aspirated for a considerable period of time (about 2 hours) until no further evolution of CO_2 can be detected by baryta water. Such blood is never free from CO_2 when tested with sulphuric acid. "CO_2 saturated blood" designates blood which has been submitted to the action of carbon dioxide until no more gas is absorbed.

The variation in the corpuscular volume of blood with varying carbon dioxide contents was described by Limbech(14) in 1894, and can be readily observed by direct experiment. The degree of change, however, is a definite function of the time. This fact is evident from the following results which show the volume of serum obtained from 100 c.c. of normal blood, and from the same blood, saturated with CO_2, after varying times.

Blood (100 c.c.)	Time after saturation	Serum	Corpuscles
Normal	—	60 c.c.	40 c.c.
CO_2 saturated	5 mins.	52 „	48 „
„	2 hours	56 „	43·4 „
„	4 „	58 „	41·6 „

In the experiments recorded a period of approximately two hours elapsed in the preparation of the different samples of blood, so that this time factor may be regarded as constant. The following figures give the corpuscular volume for four different samples of blood, and the average values are those used in the calculations given in later experiments.

Volume of serum from 100 c.c. of blood:

	CO_2 free	Normal	CO_2 saturated
(1)	67 c.c.	66 c.c.	60·5 c.c.
(2)	63 „	63·5 „	60 „
(3)	60 „	60 „	55 „
(4)	60 „	60 „	56·6 „
Average	62·5 „	62·4 „	58 „
Average volume of corpuscles (by difference)	37·5 „	37·6 „	42 „

It may be assumed that under these experimental conditions the volume of the serum from CO_2 free blood is identical with that from normal blood. Presumably during the process of abstraction of CO_2 from the normal blood any alteration in volume resulting from the removal of the CO_2 adjusts itself during the prolonged period necessary to remove the last trace of CO_2. Probably the CO_2, to which the red cell is freely permeable, increases the number of ions in the cell by the decomposition of some previously slightly ionised complex. The increased volume of the cell is caused by the rapid passage of water from the serum into the cell owing to the increased osmotic pressure and the subsequent diminution in volume is due to the slow diffusion of the increased ion into the serum until equilibrium is attained.

The total alkali and potassium of the ash of blood and serum.

Method for total alkali. It is a matter of considerable difficulty to obtain concordant results for the alkali of the ash of serum and blood. Apparently the salts of the alkalies are, to some degree, volatile at high temperatures. In view of the importance of the matter we put forward our method in detail. 20 c.c. of blood or serum were dried at 100° C. and then decomposed as completely as possible in an oven at 300° C. The carbonaceous residue was crushed as completely as possible with an agate pestle, and the platinum basin was then heated uniformly to very dull redness to decompose the residual organic matter. This operation was repeated and the ultimate residue was again very finely powdered. A small quantity of water was now added to the basin and gently warmed, and the contents were washed as completely as possible into a flask. The residue in the basin was ignited to oxidise the residual carbon, and 20 c.c. of 0·1 *N* hydrochloric acid added, the contents being again washed into the flask. In this way all the organic substances were decomposed, and all the inorganic matter was transferred to the flask without loss by volatilisation. The contents of the flask were now heated to about 100° C. in order that the salts in the interstices of any carbon particles might react with; or dissolve in the hydrochloric acid solution. When cool the whole of the contents were transferred to a 250 c.c. measuring flask, made up to the required volume with water, and left to stand for a few minutes. It was then filtered and 200 c.c. of the filtrate titrated against 0·1 *N* sodium hydroxide, using 6–8 drops of phenol phthalein as indicator.

The total alkali of the ash of blood and serum. The following results show the amounts of alkali present in the ash of (1) 100 c.c. of blood and (2) 100 c.c. of serum from the blood. The figures express the power of the ash to react with acids (such as hydrochloric acid) in terms of c.c. of normal alkali. The final result is the average of four different samples of blood.

Alkali in terms of *N* NaOH in 100 c.c. of:

	Blood	Serum
(1)	3·75 c.c.	3·78 c.c.
(2)	3·53 „	3·47 „
(3)	4·5 „	3·94 „
(4)	5·19 „	4·0 „
Average	4·24 „	3·8 „

We may express the result by saying that the ash of blood contains alkali equal to 4·24 c.c. *N* NaOH and of this the serum contains 2·39 c.c. *N* NaOH, and the corpuscles 1·85 c.c. *N* NaOH. The amount of alkali in the ash of 100 c.c. of serum (3·8 c.c. *N* NaOH) is less than that in 100 c.c. of corpuscles (4·9 c.c. *N* NaOH), although the actual quantity of alkali in the ash of serum obtained from a definite volume of blood is greater than in the ash of the corpuscles contained in the same volume of blood.

The alkali of the ash of serum obtained from (a) *normal blood,* (b) CO_2 *free blood and* (c) CO_2 *saturated blood.* The ash of serum from blood containing varying quantities of CO_2 contains variable quantities of alkali. The following figures give the quantities of alkali in the ash of serum obtained from four samples of blood, each sample of blood being divided into three parts and rendered (1) CO_2 free, (2) normal and (3) CO_2 saturated before the separation of the serum from it.

Alkali in terms of *N* NaOH contained in the ash of 100 c.c. of serum from:

	CO_2 free blood	Normal blood	CO_2 saturated blood
(1)	3·65 c.c. *N* NaOH	4·37 c.c. *N* NaOH	5·97 c.c. *N* NaOH
(2)	3·19 „	4·15 „	5·22 „
(3)	3·12 „	3·94 „	5·87 „
(4)	3·37 „	4·0 „	5·94 „
Average	3·33 „	4·125 „	5·75 „

When blood passes from CO_2 free to normal there is a 23 p.c. increase on the alkali which may be obtained from the ash of the serum; and similarly from CO_2 free to CO_2 saturated a 72 p.c. increase in the alkali of the ash of the serum.

The potassium of blood and serum. Sodium and potassium form the predominant alkali ions of blood and serum. By determining the quantity of potassium present under any condition a fair approximation to the sodium value may be obtained by difference.

The potassium estimations were made on the neutralised filtrates obtained after determining the total alkali contained in the ash of serum from any sample of blood.

These neutralised filtrates were evaporated to dryness on a water-bath. The residue was redissolved in water and filtered into a platinum dish containing 6–7 c.c. of perchloric acid solution. The remaining processes were those used in the ordinary estimations of potassium as perchlorate. The analyses show that potassium is present in small quantities only (0·116 p.c. as KCl) in sheep's blood and is approximately equally distributed between the serum and corpuscles.

	% potassium in blood	% potassium in serum
(1)	·0579	·0595
(2)	·0653	·0601
(3)	·0650	·0660
(4)	·0642	·0634
Average	·0608	·0622

The approximate equality in the amount of potassium in blood and serum suggests that the red cell is freely permeable to the potassium ion. There is certainly no indication from the analyses that the potassium ion preponderates in the corpuscle and the sodium ion in the serum, as is stated to occur in human blood by Wonach. If the potassium in the ash of serum be considered to exist as alkali and be calculated in terms of normal alkali, it is found that of the total alkali in the ash of serum 39 p.c. is due to potassium.

The influence of carbon dioxide on the distribution of potassium between corpuscles and serum. This effect is very much less than that observed with the total alkali contained in the ash of serum. The figures under A. show that the ash of serum obtained from normal blood contains a little less potassium than the ash of serum obtained from the same blood saturated with CO_2. The change is about 6 p.c.; in a corresponding experiment with the total alkali of the ash the change was 39 p.c.

% potassium from 100 c.c. of serum from blood:

A.

	Normal	Saturated with CO_2
(1)	·0604	·0637
(2)	·0634	·0674
(3)	·0720	·0766
Average	·0653	·0690

B.

	Normal	Freed from CO_2
(1)	·083	·080
(2)	·066	·064
Average	·074	·072

Similarly, when blood is freed from carbon dioxide a little potassium enters the corpuscle (cf. B.). In this case the change was about 3 p.c.; in a corresponding experiment with the total alkali of the ash the change was 23 p.c. The small effect of CO_2 on the distribution of potassium between the corpuscles and serum of blood, compared with the great effect of this gas on the total alkali of the ash suggests that sodium and not potassium enters into a specific relation with the red cell in the processes concerned in the transport of CO_2 in the blood. Probably the potassium exists in the blood as an inorganic salt only.

The chloride, phosphate and sulphate of blood and serum.

It is generally assumed that the varying quantities of alkali in the ash of serum obtained from blood containing varying quantities of CO_2, are due to the transference of chloride from the red corpuscle to the serum, or *vice versa*, and not to any migration of the sodium or potassium ion. The previous figures, however, show that there is an actual change (that is, migration) of the potassium ion and suggest that there may be a similar definite change in the sodium ion. Moreover, calculations based on the total alkali of ash of serum under varying conditions and of the chief anions (—Cl, —PO_4, —SO_4) under these conditions, show that there is a considerable transference of the sodium ion from the cell to the serum, or *vice versa*, when the CO_2 of the blood is increased or diminished.

Method of estimating chloride. 20 c.c. of 20 p.c. metaphosphoric acid were added to 20 c.c. of blood or serum diluted with water. The mixture was made up to 250 c.c., and filtered after allowing it to stand for ½ hour. 5 c.c. of concentrated nitric acid and 25 c.c. of 0·1 *N* silver nitrate were added to 150 c.c. of the filtrate, the volume being made up to 250 c.c. and left overnight. 200 c.c. of the filtrate, to which 20 c.c. of ferric indicator had been added, were finally titrated against 0·05 *N* ammonium thiocyanate.

Chloride in blood and serum. An analysis of blood and serum shows not only that much more chloride is contained in the serum than the corpuscles, but that the percentage in the serum is much greater than in the corpuscle. It was found that 100 c.c. of blood contained ·347 g. of chlorine as chloride. Of this, the serum contained ·247 g., and the cells ·100 g. In terms of percentages, the serum contained ·396 p.c., and the corpuscles ·267 p.c. of chlorine. The percentage of chlorine in whole blood, calculated as sodium chloride was low—only ·57 p.c.—although the tonicity of the blood, measured by hæmolysis in varying strengths of sodium chloride, did not differ from that of normal blood. It is of interest to observe, however, that normal blood does not hæmolyse in diminishing strengths of sodium chloride solution until the percentage has fallen to approximately ·6 p.c.

Chloride in the sera from (1) *normal blood,* (2) CO_2 *free blood,* (3) CO_2 *saturated blood.* The transference of chloride from the serum to the corpuscles under the influence of CO_2 is a statement upon which all investigators are agreed. But this transference constitutes only a small portion of the total ionic interchange between the corpuscles and serum under the influence of CO_2. The following figures give the percentages of chlorine as chloride in various sera obtained from two samples of blood.

% chlorine in serum from blood:

	CO_2 free	Normal	CO_2 saturated
(1)	·416 g.	·494 g.	·367 g.
(2)	·372 „	·367 „	·318 „
Average	·394 „	·385 „	·342 „

From these figures it is evident that when CO_2 free blood is put into equilibrium with alveolar air the percentage of chlorine in the serum diminishes ·009 p.c.; and similarly, when CO_2 free blood is saturated with CO_2 the amount of chlorine in the serum diminishes by ·052 p.c. Expressed in terms of percentages of the total quantity of chlorine present in the serum of CO_2 free blood, the loss from the serum to normal blood is 2·3 p.c.; and from the serum on passing to CO_2 saturated blood is 13 p.c. It may be observed that the variations are very much less than the variations met with in the alkali of the ash of sera obtained from corresponding types of blood.

The total phosphate in the sera from (1) *normal blood,* (2) CO_2 *free blood,* (3) CO_2 *saturated blood.* It appeared reasonable to assume that the phosphate anion might share in the migration process from the serum to the corpuscle under the influence of CO_2 in a manner similar to that observed with the chloride ion. A number of determinations were made by Neumann's method of the total phosphate contained in the different sera. The results were obtained with three different samples of blood.

Phosphate in terms of P_2O_5 contained in 100 c.c. of serum from blood:

	CO_2 free	Normal	CO_2 saturated
(1)	·0259 g.	·0248 g.	·0242 g.
(2)	·0273 „	·0274 „	·0281 „
(3)	·0309 „	·0310 „	·0308 „
Average	·0280 „	·0277 „	·0277 „

It may be observed that there is a remarkable constancy in the quantity of total phosphate in the various sera in contrast to the chlorine results in similar experiments. The results indicate that the phosphate ion does not migrate when the quantity of CO_2 in the blood is varied. The small variations which occur are probably due to variations in the degree of hæmolysis in the sera, since it is impossible to free blood from CO_2, or to saturate blood with CO_2, without rupturing some red cells. This hypothesis is supported by the fact that there is much more total phosphate (Neumann's method) in the corpuscles than in the serum of blood, as the following figures show. P_2O_5 in blood ·0476 p.c.; in serum ·0284 p.c.; in corpuscles ·0755 p.c.

The inorganic phosphate in blood. The whole of the phosphorus, whether present in organic combination or as an inorganic salt, is estimated as phosphate by Neumann's method. The above phosphate figures, therefore, may not be analogous to the chloride figures. A method was consequently adopted to estimate the inorganic phosphate present in blood and serum.

50 c.c. of blood or serum was diluted to 250 c.c. with 10 p.c. trichloracetic acid solution in a standard flask. The resultant mixture was filtered and 150 c.c. of the filtrate was treated with an excess of magnesium citrate mixture and ammonia. The precipitate thus obtained was too small for accurate manipulation, indicating that there is practically no inorganic phosphate in serum or blood. This deduction offers a simple explanation why the phosphate ion does not migrate from the serum to the corpuscle in a manner analogous to that observed with the chloride radicle under the influence of CO_2.

The sulphate of blood and serum. The only other important inorganic acid radicle which might possibly play a part in the ionic migration under the influence of CO_2 is the sulphate ion. Although the migration of this ion from serum to corpuscle has been described by De Boer(4), the quantity of inorganic sulphate present in sheep's blood or serum is too small for accurate estimation by the usual methods. If the whole of the sulphate present in serum migrated to the corpuscles under the influence of CO_2, the total error caused by the omission of this factor would be negligible.

The reversibility of the change in alkali and chloride in serum under the influence of carbon dioxide.

The reversibility of the migration of alkali and chloride from the serum to the corpuscles, or *vice versa*, under the influence of CO_2 was investigated. The following figures give the quantity of alkali in terms of N NaOH contained in 100 c.c. of serum obtained from (1) blood freed from CO_2, (2) blood saturated with CO_2, (3) blood first saturated with CO_2, and then freed from it. Similar results were given by the chloride estimations from the same sera.

Alkali in terms of *N* NaOH contained in the ash of 100 c.c. of serum from:

(1) 3·65 c.c.; (2) 5·97 c.c.; (3) 3·37 c.c.

Chloride in 100 c.c. of serum from:

(1) ·416 g.; (2) ·367 g.; (3) ·409 g.

It is clear from the above figures that the migrations of alkali and chloride from the serum to the cell, under the influence of CO_2, are completely reversible. The result appears to dissociate the possible vital activity of the red cell from the processes involved in the transport of CO_2, since saturation with CO_2 has a very toxic effect on all living matter.

The average quantities of carbon dioxide combined with blood and serum.

Normal blood and serum. The following figures give the percentage amounts of combined CO_2 in a series of bloods and corresponding sera, when put into equilibrium with alveolar air.

Percentage of carbon dioxide in:

	Blood	Serum
(1)	56·2	42·0
(2)	50·5	57·2
(3)	52·7	47·2
(4)	54·5	55·0
(5)	58·0	46·0
(6)	63·0	58·0
Average	55·8	50·9

The carbon dioxide capacities of sera obtained from (1) *normal blood,* (2) CO_2 *free blood and* (3) CO_2 *saturated blood, when put into equilibrium with alveolar air.* In the previous pages the ionic interchanges which take place between the corpuscle and the serum when the quantity of CO_2 in blood is altered, have been detailed. The sera obtained from these different kinds of blood possess very different capacities for carrying CO_2. This fact is illustrated in the following series of figures obtained from different samples of blood. All the sera were put into equilibrium with alveolar air before their CO_2 contents were determined. The figures give the combined CO_2 only.

Percentage of combined CO_2 in alveolated serum from blood:

	CO_2 free	Normal	CO_2 saturated
(1)	27·5	55	77
(2)	25	46	81
(3)	36	58	88
(4)	29·4	48·3	70·5
Average	29·5	51·8	84·1

DISCUSSION OF RESULTS.

Certain general conclusions may be drawn regarding the effects produced by carbon dioxide on the blood from the average figures given in the previous pages.

The inorganic kations and anions of blood are mainly sodium, potassium, chloride, phosphate and sulphate. From the analyses it is clear that only the sodium and the chloride ions enter to any extent in the ionic interchange. Therefore, all the effects observed are worked out in terms of these ions and the correlations are made by using the two equations:

$$(a)\ \ NaOH + CO_2 = NaHCO_3$$

$$(b)\ \ NaCl + CO_2 + H_2O = NaHCO_3 + HCl$$

according to which 1 c.c. N NaOH = 22·24 c.c. CO_2 = ·0355 g. Cl.

The alkali of the ash of various sera compared with the corresponding capacities of the sera to carry carbon dioxide.

100 c.c. serum from	(1) CO_2 capacity (alveolated)	(2) Variation in CO_2 capacity	(3) Total alkali of ash as N NaOH	(4) CO_2 equivalent of NaOH	(5) Variation in CO_2 cap. of ash
Normal blood	51·8 %	22·3 %	4·125 c.c.	92 c.c.	16·7 c.c.
CO_2 free blood	29·5 ,,	54·6 ,,	3·33 ,,	75·3 ,,	52·7 ,,
CO_2 saturated blood	84·1 ,,	48·5 ,,	5·75 ,,	128·0 ,,	43·7 ,,
CO_2 saturated blood (corr. for vol.)	78·0 ,,		5·33 ,,	119·0 ,,	

It is evident from these figures that the carbon dioxide equivalent of the ash (col. 4) is much greater than the carbon dioxide carrying power of the corresponding serum when put into equilibrium with alveolar air (col. 1). But the change in the carbon dioxide equivalent of the ash of the different sera (col. 5) is approximately equal to the change in the carbon dioxide carrying powers of these sera (col. 2) when the quantity of this gas in the blood from which they have been obtained is varied. This constant relation indicates that in the serum the carbon dioxide is carried as sodium bicarbonate.

The difference between the carbon dioxide equivalent of the ash and the capacity of the corresponding serum to carry carbon dioxide.

100 c.c. serum from	CO_2 capacity (alveolated)	CO_2 equivalent of ash	Difference
Normal blood	51·8 c.c.	92·0 c.c.	40·2 c.c.
CO_2 free blood	29·5 ,,	75·3 ,,	45·8 ,,
CO_2 saturated blood	84·1 ,,	128·0 ,,	43·9 ,,
CO_2 saturated blood (corr. for vol.)	78·0 ,,	118·5 ,,	40·5 ,,

It may be seen that the CO_2 equivalent of the ash always exceeds the CO_2 capacity of the corresponding serum, although these CO_2 capacities vary so widely as 29·5 p.c. and 78·0 p.c. This difference is represented by some organic sodium salt in the serum. The quantity of sodium thus combined is considerable (approximately equal to that combined with CO_2 in the serum of normal blood). Possibly this sodium is combined with the protein of the serum and constitutes a second reserve of alkali (in addition to that supplied by the red blood corpuscles) which may be drawn upon when the CO_2 of blood is increased in quantity. This suggestion is supported by the fact that the excess of alkali in the ash (last column) progressively diminishes as we pass from CO_2 free blood to normal blood, and from normal blood to CO_2 saturated blood. Serum proteins, therefore, act not only as a source of alkali reserve but also as a means of preserving the neutrality of the blood.

The change in the chloride concentration compared with the change in the carbon dioxide capacity of the corresponding serum.

100 c.c. serum from	% of Cl	Change in Cl conc.	CO_2 equiv. of Cl change	CO_2 capacity of serum	Difference
Normal blood	·382	·012 g.	7·5 c.c.	51·8 c.c.	22·3 c.c.
CO_2 free blood	·394	·052 „	32·5 „	29·5 „	54·6 „
CO_2 saturated blood	·342	·076 „	49·0 „	84·1 „	48·5 „
CO_2 saturated blood (corr. for volume)	·318			78·0 „	

It is evident from these figures that the passage of chloride from the serum to the corpuscles under the influence of carbon dioxide accounts for only one-third of the increased capacity of the serum to carry this gas on passing from CO_2 free blood to normal blood. On saturating blood with carbon dioxide a much greater quantity of chlorine enters the corpuscles. Under these circumstances the change in the concentration of the chloride ion accounts for the whole of the increased carbon dioxide capacity of the serum obtained from CO_2 saturated blood.

The effect of an altered distribution of alkali between the corpuscles and serum on the capacity of the blood to carry carbon dioxide. The sera obtained from CO_2 free blood and CO_2 saturated blood possess widely different capacities to carry CO_2 when put into equilibrium with alveolar air. This variation in CO_2 capacity has been shown to be accompanied by similar variations in the alkali content of the corresponding sera and corpuscles. Thus, in CO_2 free blood there is a large quantity of alkali in the ash of the corpuscles and a small quantity in the ash of the serum, whilst in CO_2 saturated blood there is a small amount of alkali in the ash of the corpuscles and a large amount in the ash of the serum. It is of interest to observe that these large differences are not associated with any change in the total carbon dioxide carrying powers of the two constituents of each type of blood. Thus, the following figures were obtained:

100 c.c. of CO_2 free blood were separated into 62·4 c.c. of serum and 37·6 c.c. of corpuscles. The serum put into equilibrium with alveolar air combined with 43 p.c. of CO_2. Therefore, 62·4 c.c. of serum contained 26·8 c.c. of CO_2. The corpuscles suspended in ·85 p.c. NaCl combined with 45 c.c. of CO_2. Therefore, the total CO_2 held in combination by the corpuscles and serum of the original blood was 71·8 c.c. of CO_2.

100 c.c. of the same blood was saturated with CO_2 and then separated into 58 c.c. of serum and 42 c.c. of corpuscles. The serum, put into equilibrium with alveolar air, combined with 86 p.c. of CO_2. Therefore, 58 c.c. of serum contained 50 c.c. of CO_2.

The corpuscles suspended in ·85 p.c. NaCl combined with 21 c.c. of CO_2. Therefore, the total CO_2 held in combination by the corpuscles and serum of the original blood was 71 p.c. of CO_2.

The equality of these results shows that it is a matter of indifference whether the available alkali is held in the serum or corpuscles as far as the capacity to carry CO_2 is concerned when put into equilibrium with alveolar air. It may, therefore, be assumed that if the CO_2 is present in the serum as sodium bicarbonate a similar fact holds true for the CO_2 in the corpuscle. This deduction implies that the hæmoglobin of the red cell does not combine with carbon dioxide.

SUMMARY.

(1) The increased volume of the corpuscles of blood with increased carbon dioxide content is a definite function of the time. This indicates that carbon dioxide increases the number of ions in the cell by the decomposition of some slightly ionised compound, and that the liberated ion slowly diffuses across the envelope of the red cell.

(2) There is a 23 p.c. increase in the alkali of the ash of serum on passing from CO_2 free blood to normal blood; and a 72 p.c. increase in the alkali of the ash of the serum on passing from CO_2 free blood to CO_2 saturated blood.

(3) Potassium is contained in small quantities only in sheep's blood (·116 p.c. as KCl). Under the influence of carbon dioxide the transference of potassium to the cell or from the cell is small (about 5 p.c.). Probably, potassium exists in blood as an inorganic salt only, and does not enter into any specific relation with the contents of the red cell.

(4) The serum loses 2·3 p.c. of its total chloride (expressed as chlorine) when blood passes from CO_2 free to normal blood; and 13 p.c. of its total chloride on passing from CO_2 free to CO_2 saturated blood.

(5) There is only a trace of inorganic phosphate in sheep's blood. No appreciable change takes place in the phosphate content of serum under the influence of CO_2.

(6) The quantity of inorganic sulphate in sheep's serum is too small for accurate estimation by the ordinary methods.

(7) There is a large difference between the alkali of the ash and the capacity of the corresponding serum to carry CO_2. This represents an organic sodium compound in the serum—possibly protein—and constitutes a source of reserve alkali when the quantity of carbon dioxide in the blood is increased.

(8) The differences in the alkali of the ash of different sera prepared from the same blood is approximately equal to the differences in the carbon dioxide carrying powers of these sera. This constant relation indicates that carbon dioxide is carried as sodium bicarbonate in the serum.

(9) The change in the chloride concentration of the serum accounts for one-third of the change in the carbon dioxide carrying power of the serum on passing from CO_2 free blood to normal blood. Hence, sodium must pass freely from the red cell to the serum when the tension of carbon dioxide to which the blood is subjected varies from 0 to 5 p.c. of an atmosphere. The whole of the change in the carbon dioxide carrying power is, however, accounted for by the change in the chloride concentration on passing from CO_2 free blood to CO_2 saturated blood.

(10) The total quantity of carbon dioxide carried by the cells and serum of CO_2 free blood is the same as that by the cells and serum of CO_2 saturated blood, although the distribution of alkali between the cells and serum attains a maximum difference in these two kinds of blood. This shows that carbon dioxide is carried by the cells in the same way as in the serum—as sodium bicarbonate—and that the hæmoglobin of the red cell does not combine with carbon dioxide.

(11) The large effects produced by carbon dioxide on the distribution of sodium and chlorine between the serum and corpuscles compared with the small effect produced by it on the distribution of potassium, indicates that sodium and chlorine enter into a specific chemical relation with some constituent of the red cell, which is not shared by the other ions of the blood.

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