THE CARBON DIOXIDE CARRYING POWER OF THE CONSTITUENTS OF PLASMA. THE ALKALI RESERVE OF BLOOD.

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CONTENTS.

THE hypotheses put forward to elucidate the relations of carbon dioxide in blood may be briefly summarised into three categories: (*a*) that carbon dioxide is associated with the diffusible and non-diffusible alkali of blood (Loewy and Zuntz(1)); (*b*) that a considerable portion of the carbon dioxide is in combination with hæmoglobin (Bohr(2), Buckmaster(3)); and (*c*) that carbon dioxide is present in blood entirely in the form of bicarbonate (Haldane, Hasselbalch(5), Parsons(6)). The last hypothesis has recently been analysed from a mathematical standpoint by Parsons(6) on the assumption that the laws of mass action may be applied to mixtures of colloid and crystalloid substances contained in a fluid so complex as blood. On this assumption he compared the carbon dioxide dissociation curve of a solution of sodium bicarbonate with that found by Christiansen, Douglas and Haldane(4) for blood. He concluded that the whole of the combined carbon dioxide of blood is present in the form of sodium bicarbonate and that the proteins of blood confer on this bicarbonate the property of being an efficient carrier of carbon dioxide. The fundamental basis of the bicarbonate hypothesis is that the proteins of blood act as weak acids, and by virtue of this property cause sodium bicarbonate to dissociate at tensions of carbon dioxide present in alveolar air. This hypothesis therefore is complementary to that put forward by Moore(7) that the maintainance of the neutrality of blood is one of the main functions of the protein of blood. But

Bayliss(8) observed that the proteins of plasma do not function as acids or alkalies at hydrogen ion concentrations compatible with life. Therefore the bicarbonate hypothesis, put forward to explain the carbon dioxide carrying power of blood, must be modified to the extent that the second weak acid which shares the sodium with the carbon dioxide must be associated with the corpuscular elements of the blood. In a later communication Parsons(9) states that the protein of blood which confers on bicarbonate the capacity of being an efficient carrier of carbon dioxide is mainly, if not entirely, hæmoglobin. This hypothesis, that hæmoglobin functions as an acid capable of decomposing sodium bicarbonate, is directly opposed to the conclusions of Buckmaster(3) that the transport of carbon dioxide is effected mainly by hæmoglobin.

Methods. The experimental results were obtained by determining the capacity of blood and its constituents to combine with carbon dioxide. The capacity of any fluid to carry carbon dioxide was found by exposing it to human alveolar air and then determining the total quantity of carbon dioxide contained in it by van Slyke's method. In the results submitted the amount of carbon dioxide in solution is not included since the object of the experiments was to determine the manner in which carbon dioxide is combined in blood. In the case of fluids carrying a small quantity of carbon dioxide in combination the amount dissolved forms a considerable portion of the whole and gives an erroneous impression of magnitude. In many cases the plasma fractions were ashed and the capacity of the ash, when dissolved in water, to combine with carbon dioxide was determined. In regard to these experiments it may be observed that the values so obtained give the maximum quantity of carbon dioxide which could be carried by the alkaline salts in the original fluid. It is clear that the organic salt of a metal of the alkali group, although incapable of carrying carbon dioxide in the original fluid, yet when ashed yields free alkali capable of combining with carbon dioxide.

The permeability of the red blood corpuscles.

The ash of serum. The bicarbonate hypothesis for the transport of carbon dioxide in the blood depends upon the assumption that a ready interchange of anions or kations takes place across the envelope of the red blood corpuscles. The fact that red blood corpuscles circulate in the vascular system in a fluid medium has led to the assumption that such an interchange of ions takes place across the cell envelope. In the case of the gases of blood, oxygen

and carbon dioxide, the fact is self-evident, but with regard to inorganic anions and kations as Cl, P_2O_5, K.Na, etc., the proofs of free interchange are not readily obtained. Hamburger(10) stated that red blood corpuscles were permeable to Cl and P_2O_5, even in isotonic solution, and that when CO_2 was added to blood Cl passed from the serum to the corpuscles to preserve the neutrality of blood. Höber(11), on the other hand, stated that under normal conditions the red blood corpuscle is impermeable to anions, and permeability is produced only by treatment with CO_2. Gürber found that the red blood corpuscles were impermeable to K and Na, and his results were confirmed in 1904 by Höber(12). In 1909 Hamburger and Hekma(13) stated that small quantities of Ca may penetrate into red blood corpuscles, but Abderhalden and Fränkel state that Ca is found only in the plasma. Recently de Boer(14) has demonstrated the passage of small quantities of SO_4 from the plasma into the corpuscles. It may be concluded, therefore, from the results of experiments on the permeability of red blood cells to anions and kations that interchange cannot be demonstrated with ease and certainty. The fact that no such interchange readily occurs can be shown by a simple experiment. On the addition of red blood cells to sodium chloride solutions of varying strengths hæmolysis usually occurs at ·55 p.c. NaCl. The experiment may be repeated in another form, thus, a drop of blood is added to 10 c.c. of ·9 p.c. NaCl; after half an hour water is added to the mixture to bring the salt content to ·8 p.c. NaCl. A similar addition of water reducing the NaCl content ·1 p.c. is repeated every half hour. It is found that hæmolysis occurs at ·55 p.c. NaCl in this case also. If any interchange of anions or kations took place across the surface of the corpuscle it is clear that hæmolysis would occur with a lower concentration of sodium chloride in the second case—in fact, with perfect interchange the corpuscles could be obtained intact in pure water. In the section dealing with the ash of serum obtained from normal blood and from blood freed from CO_2 it is evident that under these conditions also no Na or Cl passes from the serum to the corpuscle or from the corpuscle to the serum. The strength of the envelope of the red blood corpuscle, shown by the low percentage of NaCl (·55 p.c.) required to produce hæmolysis, indicates that conditions may arise when the contents of the corpuscle are not in equilibrium with the plasma. Therefore the general consideration of the permeability of the red blood corpuscle to anions and kations does not lead to conclusions favourable to the bicarbonate hypothesis which postulates that hæmoglobin functions as

an acid and thereby shares the available sodium of the blood with carbon dioxide.

(*a*) Normal serum. Experiments have been made to determine the relative capacities of serum and the inorganic salts contained in it to carry carbon dioxide. In order to obtain a solution of the inorganic salts a known volume of serum was dried and ashed at a dull red heat. The ash was dissolved in a volume of water equal to that of the original serum. The solution was exposed to alveolar air until equilibrium was attained and the amount of carbon dioxide contained in it was compared with that obtained from the original serum similarly treated. The following figures give the values obtained in two typical experiments:

Cat's blood	Serum (alveolated)	46·3 % CO_2
	Solution of ash (alveolated)	46·2 % „
Rabbit's blood	Serum (alveolated)	32·1 % „
	Solution of ash (alveolated)	32·4 % „

The identity of the values giving the capacity of serum and of the inorganic salts contained in it to carry carbon dioxide appears to offer strong evidence in favour of the hypothesis that all the carbon dioxide of blood exists in combination with sodium. The acceptance of this hypothesis from the above figures would, however, demand that no organic acid such as lactic acid should exist in the serum in combination with sodium. That such a condition should exist is improbable—in fact, later experiments show that serum always contains lactic acid.

(*b*) The ash of serum obtained from blood containing no carbon dioxide. Parsons' hypothesis that the sodium of blood is shared between the two weak acids hæmoglobin and carbon dioxide according to the law of mass action can be readily tested by experiments similar to those quoted for serum. On this hypothesis blood freed from carbon dioxide should have all its available alkali combined with the hæmoglobin. Since the corpuscles can be removed by centrifuging the serum from CO_2-free blood should contain much less inorganic salt capable of combining with carbon dioxide than serum obtained from blood containing a normal quantity of carbon dioxide.

	I	II
Serum from normal blood:		
Capacity of solution of ash to carry carbon dioxide ...	43·0 %	46·2 %
Serum from CO_2 free blood:		
Capacity of solution of ash to carry CO_2	42·0 %	44·6 %

It is evident from these figures that on depriving blood of carbon dioxide the sodium does not leave the serum to enter into combination with the hæmoglobin contained in the corpuscles. These results have been explained by assuming that when carbon dioxide is removed from

blood a constant reaction is preserved, not by the entrance of sodium into the corpuscle, but by the exit of chlorine from them into the plasma. This explanation accords with the statement of Hamburger(10) that the red blood corpuscle is permeable to the Cl ion. This hypothesis is disproved by two facts—(1) the constancy of the available alkali contained in the ash of serum obtained from normal blood and from CO_2-free blood, and (2) the identity of the Cl content of serum obtained from normal blood and CO_2-free blood. (i) The figures above give the alkali contained in the ash of serum available for combining with CO_2 and not the total sodium. Whether the sodium ion passes into the corpuscle from the serum or the chlorine ion passes into the serum from the corpuscle, the alkali contained in the ash of serum available for combining with CO_2 would be diminished to the same degree. The available alkali contained in the ash of normal serum is identical with that in the ash of serum obtained from CO_2-free blood. (ii) Direct experiment shows that there is no difference in the chlorine content of serum of normal blood and serum obtained from CO_2-free blood. The relative quantities of chlorine present in the serum of normal blood and in the serum from CO_2-free blood were in the ratio of 100 to 95. The two figures are equal within the limits of experimental error. The comparative results obtained by analysing the ash of normal serum and that obtained from CO_2-free blood offer no evidence in favour of the hypothesis that hæmoglobin acts as a weak acid and shares with CO_2 the available free alkali in the blood. The results can be explained on the bicarbonate hypothesis only by assuming that the proteins of blood plasma, and not the hæmoglobin contained in the red blood corpuscle, function as the second weak acid. This alternative hypothesis is considered in the experiments dealing with the capacity of serum proteins, obtained from normal and CO_2 free serum, to combine with CO_2.

Lactic acid in blood. The presence of lactic acid in blood has been demonstrated by many observers. Probably the most accurate experiments on this subject are those of Ryffel(15), who found that resting human blood contained ·012 p.c. lactic acid whilst blood drawn after exercise contained ·07 p.c. lactic acid. The question was investigated to determine (i) the cause of the steady diminution in the CO_2 capacity of shed blood, (ii) the change which occurs in blood after the removal of CO_2, as evidenced by a diminished capacity to recombine with CO_2, and (iii) the effect of adding lactic acid to blood on its capacity to carry CO_2.

(i) Changes in the carbon dioxide capacity of drawn blood. Christiansen, Douglas and Haldane(4) have drawn attention in

their paper (p. 246) to the fact that the capacity of blood to carry carbon dioxide diminishes after removal from the body. They offer no explanation of this fact. The degree to which shed blood loses its capacity to carry carbon dioxide is illustrated by the following experimental results:

Interval after removal from animal	CO_2 capacity
·5 hour	49·4 % CO_2
1·5 hours	46·8 % „
3·5 „	38·0 % „
5·5 „	32·0 % „

This steady fall in carbon dioxide capacity might be due to the production of lactic acid in blood comparable to that produced in dying tissues. The quantity of lactic acid in blood was therefore determined at different intervals of time. Considerable variations in the quantity of lactic acid in fresh blood have been observed. The following figures show typical results:

	A	*B*
Fresh blood	·07 % lactic acid	·1 % lactic acid
Blood after 3 hours ...	·18 % „ „	·133 % „ „

The blood used in the above experiments was obtained from anæsthetised cats. The lactic acid values for fresh blood in cats (*A*) and (*B*) presumably represent the different degrees of asphyxia induced by the anæsthetic in the two cases. In both cases, (*A*) and (*B*), the lactic acid present in the blood augmented on standing, indicating that blood is like every other tissue of the body in so far that lactic acid is produced in it on dying. It may be observed that ·1 p.c. lactic acid is capable of combining with sufficient sodium bicarbonate to diminish the CO_2 capacity of blood 24 p.c. Therefore the production of lactic acid in dying blood is capable of accounting for the steady diminution in the carbon dioxide capacity of blood after removal from the body.

(ii) The diminished capacity of serum obtained from CO_2-free blood to combine with CO_2. Serum obtained from CO_2-free blood combines with less CO_2 than serum obtained from the same blood from which the CO_2 has not been abstracted, thus:

	A	*B*
Serum from normal blood (alveolated)	33 % CO_2	46·3 % CO_2
Serum from CO_2-free blood (alveolated)	22 % „	20 % „
Diminution in CO_2 capacity	11 % „	26·3 % „

The diminished capacity of serum obtained from CO_2-free blood to carry carbon dioxide, although the ash of this serum contains just as much alkali as the ash of serum from normal blood (*vide supra*) might be due to the production of lactic acid in blood freed from CO_2 combining with the sodium bicarbonate and so rendering the sodium unavail-

able for carrying CO_2. The plausibility of this suggestion is indicated by the following lactic acid determinations:

	CO_2 capacity	% of lactic acid
Serum from normal blood (alveolated)	33 %	·1 %
Serum from same blood after freeing from CO_2	22 %	·173 %

Experimentally, *in vitro*, ·073 p.c. lactic acid, if entirely combined with $NaHCO_3$, diminishes the carbon dioxide carrying power of a fluid to which it is added 18·4 p.c. In the above experiment the increase in the lactic acid content of blood to this extent diminished the CO_2 capacity of the serum by 11 p.c. only. The production of lactic acid in blood in which the hæmoglobin is saturated with oxygen brings this phenomenon into opposition with the results observed by Fletcher and Hopkins in frog's muscle.

The production of lactic acid in blood affords an explanation of the more ready removal of CO_2 from the blood than from serum, and incidentally shows that the corpuscles constitute the source of the lactic acid. In every sample of cat's blood investigated the entire CO_2 was removed by CO_2-free air. In the case of serum CO_2 could not be removed by CO_2-free air. The following figures illustrate the effect of CO_2-free air on serum:

Serum (alveolated)	37·1 % CO_2	
Serum (air free from CO_2 for four hours) (*)...	7·6 % „	(residual CO_2)
(*) alveolated	33 % „	

These figures show two facts: (i) CO_2 cannot be removed from serum by CO_2-free air, and (ii) after the removal of as much CO_2 as possible, the serum can be re-alveolated to practically the same value as before. These facts correspond with those observed in the production of lactic acid in serum. Serum, from which all the corpuscles have been removed, does not produce lactic acid. This results in (i) a considerable quantity of CO_2 remaining in the serum as Na_2CO_3 after air free from CO_2 has been pulled through it for four hours, and (ii) the serum after such treatment being able to recombine with practically as much CO_2 as the original serum. Further, the results show that the corpuscles give rise to the lactic acid produced in shed blood. The origin of lactic acid in shed blood from corpuscles affords a simple explanation of the phenomena originally observed by Pflüger, that the entire CO_2 can be removed from blood but that with serum this result cannot be attained. Incidentally the ultimate substance which acts as an acid in blood which is not found in serum is not the corpuscles as suggested by Pflüger, nor the hæmoglobin as has been assumed at a later date, but the lactic acid produced

by the formed elements of the blood. On the assumption that serum exposed to CO_2-free air for four hours contains Na_2CO_3 only the above figures show that the serum under consideration contained 15·2 p.c. CO_2 in combination with Na as $NaHCO_3$ and 22 p.c. CO_2 combined with the proteins. The figures given by Loewy are 18·8 CO_2 with the bicarbonate system and 11·8 p.c. CO_2 with the colloidal indiffusible substances in the blood.

(iii) The effect of adding lactic acid to blood on its capacity to carry carbon dioxide. The above results, illustrating the formation of lactic acid in shed blood and the change in the CO_2 capacity of such blood, suggested that the addition of lactic acid would not diminish the capacity of blood to carry carbon dioxide to such a degree as would be demanded by the bicarbonate hypothesis. To test this assumption varying quantities of lactic acid were added to fresh defibrinated blood and the CO_2 capacities of the sera obtained from the various fractions determined. The following results were obtained:

	Amount of lactic acid added to original blood	CO_2 capacity of serum
Fresh blood	0	33 % CO_2
" "	·1 %	27·3 % "
" "	·2 %	18·0 % "
" "	·3 %	13·0 % "

As previously stated, the addition of ·1 p.c. lactic acid to blood on the bicarbonate hypothesis should diminish its capacity to carry CO_2 approximately 24 p.c. From the figures it is evident that no such change in the CO_2 capacity of the serum was observed. Therefore the lactic acid must have united not with the alkaline salts only but with some other constituent of the blood, presumably the protein. The nature of this combination of the lactic acid and the protein is essentially labile since after removing the cells and protein from the blood the quantities of lactate radical present in the filtrates were as follows:

	Lactic acid
Original fresh blood to which no lactic acid had been added ...	·1 %
Blood to which ·1 % lactic acid had been added...	·2 %
Blood to which ·2 % lactic acid had been added...	·306 %
Blood to which ·3 % lactic acid had been added...	·4 %

Allowing for ·1 p.c. lactic acid contained in the original blood it is evident that no added lactic acid was removed by the corpuscles or coagulated protein. On the bicarbonate hypothesis the addition of ·13 p.c. lactic acid to the blood should have neutralised all the bicarbonate and have prevented the serum from carrying any CO_2 except in solution. The discrepancy between the results demanded by the bicarbonate hypothesis and the results actually obtained are evident from the

above figures. Incidentally they show one or both of two things, (*a*) either CO_2 is carried other than as sodium bicarbonate, or (*b*) lactic acid added to blood is not neutralised by bicarbonate only but by other substances in blood, *i.e.* protein. Both these possibilities ultimately lead to the same conclusion—that the protein of blood can combine with acids, lactic acid or carbonic acid, and that the resulting complex is of an essentially labile nature.

The capacity of proteins of blood to carry carbon dioxide.

(i) Fibrinogen. Many phenomena observed in the coagulation of blood indicate that carbon dioxide may enter into intimate relations with fibrinogen. Among such phenomena may be mentioned (*a*) the coagulation of peptone blood by carbon dioxide, (*b*) the adjuvant effect of carbon dioxide on the production of intravascular coagulation by the injection of thrombokinase, and (*c*) the titratable alkalinity of blood is diminished after coagulation (Loewy and Zuntz; v. Limbech). A series of experiments were therefore carried out to determine the effect of coagulation on the alkali reserve of blood, plasma, and fibrinogen.

The blood of an anæsthetised cat was obtained directly from the carotid artery. To a portion of it potassium oxalate was added to the extent of ·1 p.c. A second portion was allowed to clot and the fibrinogen was removed by whipping. The oxalated blood and the defibrinated blood were then alveolated and their CO_2 content determined.

Oxalated blood	58 % CO_2
Defibrinated blood	53·6 % „

A control experiment showed that ·1 p.c. potassium oxalate had no effect on the capacity of blood to carry carbon dioxide. The capacity of blood to combine with carbon dioxide was definitely diminished by the coagulation and removal of fibrin.

An experiment similar to the above was carried out on plasma. Potassium oxalate to the extent of ·1 p.c. was added to the blood of a cat and oxalate plasma obtained by centrifuging. A portion of this plasma was clotted by the addition of a trace of fibrin ferment and the serum obtained after removal of the coagulum. The carbon dioxide contents of the plasma and serum were compared after both fluids had been exposed to alveolar air.

Oxalated plasma	51·1 % CO_2
Serum obtained from plasma ...	44·0 % „

These results, also, show that the capacity of the fluid of blood to carry carbon dioxide is diminished by the coagulation and removal of fibrin.

This series of experiments was completed by determining the capacity of a solution of fibrinogen to carry carbon dioxide. Fibrinogen was obtained from oxalated plasma by a method which has been previously described by one of us(16). 25 c.c. of plasma, obtained from oxalated ox blood, were added to 225 c.c. of distilled water, and the resulting fluid brought to the isoelectric point for globulin by the cautious addition of ·1 p.c. acetic acid. The precipitate of globulin was allowed to settle and then obtained as a compact mass by centrifuging. The fibrinogen was dissolved in 25 c.c. of NaCl 1 p.c. forming a clear solution which readily coagulated on the addition of fibrin ferment. The capacities of the fibrinogen solution and of the fluid remaining after coagulation of the fibrinogen and removal of the clot to combine with carbon dioxide were determined.

Fibrinogen in 1 % NaCl (alveolated) ...	...	9 % CO_2
Fluid after removal of fibrin (alveolated)	...	3 % „

Whatever view is taken as regards the globulin of plasma, *i.e.* whether it is regarded as consisting of fibrinogen only which on coagulation splits into fibrin and serum globulin, or whether fibrinogen and serum globulin are considered to be present in plasma, it is clear that fibrinogen dissolved in neutral salt solution combines with carbon dioxide. On the assumption that the only globulin present in plasma is fibrinogen, and taking the amount present in ox plasma as ·5 p.c., the above figures show that a gram molecular weight of carbon dioxide is carried by approximately 1250 grams of fibrinogen. The amount of fibrinogen which dissolves in a gram molecular weight of acid or alkali is approximately 16,000 grams. It is necessary to assume therefore that the association of carbon dioxide with fibrinogen is not of the same kind as that involved in the solution of globulin by acids and alkalies. The results indicate that carbon dioxide is adsorbed by fibrinogen rather than that a chemical relation exists between the two substances.

(ii) Serum protein from normal blood. The experimental results indicating that carbon dioxide may be associated with fibrinogen demanded that a similar investigation should be made on the protein of serum. The initial difficulty was to obtain the proteins of serum free from salt without decomposing the labile protein complex. Bayliss(8) freed serum from salt by dialysis and found that the resultant fluid possessed no capacity to carry carbon dioxide. The objection to this method is the prolonged dialysis which is necessary to free serum from diffusible substances. Precipitation of the proteins by neutral salts also appeared to be inadmissible owing to the large quantity of neutral

salt required, and the difficulty of freeing the precipitate from the precipitating salt. A method previously described by one of us(17) of precipitating the serum by cold alcohol was adopted. This method consists in cooling absolute alcohol to − 10° C. by a mixture of ice and salt and adding to the cooled alcohol an equal volume of serum cooled to 0° C. The mixture is kept at − 10° C. for ten minutes after which the precipitated protein is obtained as a compact mass by a Martin's centrifuge. The precipitate is washed once with absolute alcohol and then twice with ether. After this it is air-dried and dissolved in water. The protein dissolves readily in water forming a solution very similar in appearance to that of the original serum. As evidence of the small amount of change suffered by the proteins in this procedure it may be stated that if plasma be used as the original fluid the solution obtained after alcohol precipitation, etc., coagulates on adding a trace of fibrin ferment. The protein precipitated from human serum by cold alcohol, dissolved in a volume of water equal to that of the original serum, combined with 21·3 p.c. CO_2 when exposed to alveolar air. This protein was associated with a considerable quantity of inorganic salts. To determine the relative quantities of carbon dioxide associated with the protein and the salts contained in the precipitated complex a similar experiment was done on another sample of the same serum in which the protein precipitate was ashed. The ash was dissolved in a volume of water equal to that of the original serum. This fluid combined with 15·5 p.c. CO_2. Therefore, assuming that all the alkali contained in the ash capable of carrying carbon dioxide existed in the protein precipitate in an equally free manner, the figures show that 5·8 p.c. CO_2 was associated with the protein independently of any alkaline salt which might have been contained in its complex. Incidentally the results show that sodium bicarbonate does not exist free in serum since 50 p.c. alcohol at − 10° C. does not precipitate a ·2 p.c. solution of sodium bicarbonate. Evidently the alkaline salts contained in the protein precipitate were present in the serum associated with the protein complex. The above experiment has been repeated with many sera. The following figures illustrate the results obtained from cats' serum:

Serum protein	22·5 % CO_2
Ash of protein	11·0 % „
Minimum quantity of CO_2 associated with protein	11·5 % „

The experimental results indicate that not only fibrinogen, but also the other proteins of plasma combine with carbon dioxide.

(iii) Serum protein obtained from serum saturated with carbon dioxide and serum freed from carbon dioxide. On the hypothesis that serum protein acts as a weak acid and thereby confers on sodium bicarbonate the function of being an efficient physiological carrier of carbon dioxide it is clear that protein obtained from serum saturated with CO_2 should contain the minimal number of sodium ions in its complex whilst protein obtained from serum freed from CO_2 should be fully loaded with sodium ions. In the former case such protein dissolved in water should possess a capacity to carry carbon dioxide much greater than that of protein obtained from CO_2-free serum. To test this hypothesis two portions of the same serum were taken. The first portion was saturated with carbon dioxide. After saturation it was found to contain 152 p.c. of carbon dioxide. The second portion had CO_2-free air pulled through it for four hours. At the end of that time its CO_2 content was reduced to 8 p.c. Both these serum fractions were precipitated by 50 p.c. alcohol at $-10°$ C., and the protein precipitated from each was dried, in the way previously described. In each case the dried precipitate was dissolved in a volume of water equal to that of the original serum. The quantities of CO_2 contained in each of these solutions after being submitted to alveolar air were now determined with the following results: Protein precipitated from serum saturated with CO_2 combined with 18·4 p.c. CO_2 when dissolved in a volume of water equal to that of the original serum. Protein precipitated from serum partially freed from CO_2 when dissolved in a volume of water equal to that of the original serum combined with 17·4 p.c. CO_2.

The practical identity of the capacity to carry carbon dioxide of protein obtained from (*a*) serum saturated with CO_2 and (*b*) serum freed from CO_2 indicates that proteins do not function as acids in the bicarbonate system of serum and that the amount of sodium contained in the protein complex does not vary with the quantity of CO_2 contained in the serum.

Remarks.

Direct evidence against the hypothesis that hæmoglobin or the proteins of blood function as weak acids capable of sharing the available alkali of blood with CO_2 has been given in the foregoing pages. This evidence consists of (*a*) the equality of the available alkali contained in the ash of serum obtained from CO_2-free blood and normal blood, (*b*) the equality of the available alkali contained in the ash of protein precipitated from CO_2-free serum and normal serum, and (*c*) the effect

of adding lactic acid to blood. These experimental results extend the observations of Buckmaster(18), who found that CO_2 cannot be liberated from $NaHCO_3$ or Na_2CO_3 by hæmoglobin or any constituent of defibrinated blood, or dialysed laked blood. The results indicate that the association of CO_2 with protein is essentially labile and of the nature of adsorption.

We may add some remarks on the arguments in favour of the bicarbonate hypothesis based upon the assumption that hæmoglobin can function as an acid in blood. (i) The isoelectric point for hæmoglobin is lower than the P_H of blood. But if the isoelectric points of other substances in blood be considered it is found that the proteins and amino acids of plasma are relatively more acidic than oxyhæmoglobin. On this argument the proteins of plasma would occupy a stronger position in the bicarbonate hypothesis than hæmoglobin. (ii) Blood, but not serum, can be freed from CO_2 by exposure to a vacuum. The experiments recorded indicate that the production of lactic acid by the corpuscles of blood is the main factor in expelling the entire CO_2 from blood when exposed to a vacuum. (iii) The oxidation of blood results in the evolution of CO_2 and increases its hydrogen ion concentration. The assumptions that the increased acidity of blood on oxidation is due to changes in the hæmoglobin, and that the evolution of CO_2 is due to this cause are not founded on experiments in which the only substances involved are hæmoglobin, oxygen and sodium bicarbonate. The peroxidase capacity of hæmoglobin, the production of lactic acid in cells in the absence of oxygen, the oxidative removal of lactic acid, the influence of the gases of the blood on the permeability of the red cells are factors which might produce the effects observed.

Summary.

(1) Lactic acid is produced from the corpuscles in shed blood. This explains: (*a*) the steady fall in the CO_2 of shed blood; (*b*) the power to abstract CO_2 from blood and not from serum by a CO_2 vacuum, and (*c*) the diminished capacity of blood, from which CO_2 has been removed, to recombine with CO_2.

(2) The addition of lactic acid to blood *in vitro* diminishes its capacity to carry CO_2 by less than 50 p.c. of the quantity demanded by the bicarbonate hypothesis. The association of lactic acid with protein is essentially labile since on coagulation of the protein all the lactate radicle is present in the filtrate.

(3) Solutions of the ash of sera obtained from (*a*) normal blood, and

(*b*) the same blood freed from CO_2 combine with equal volumes of CO_2. There is no evidence that any ionic interchange takes place between the corpuscles and serum on freeing blood from CO_2.

(4) Solutions of serum proteins obtained from (*a*) serum freed from CO_2, and (*b*) serum saturated with CO_2 combine with the same volume of CO_2. There is no evidence that the proteins of serum associate with Na when CO_2 is removed from blood.

(5) The coagulation of fibrinogen contained in any fluid—blood, plasma or a fibrinogen solution—diminishes the capacity of that fluid to carry CO_2. Quantitative results indicate that the association of CO_2 with fibrinogen is of the nature of adsorption.

(6) Solutions of protein obtained from serum by alcohol precipitation combine with more CO_2 than can be calculated as existing in combination with the alkaline salts contained in the precipitated complex.

(7) These results indicate: (*a*) that the bicarbonate hypothesis is inadmissible since neither hæmoglobin nor serum proteins function in blood as weak acids sharing the available Na with the CO_2 according to the requirements of the organism; (*b*) that the proteins of plasma adsorb CO_2 which in the case of fibrinogen is relatively large in amount; (*c*) that CO_2 is combined in blood in two ways, (i) adsorbed by the proteins and (ii) as $NaHCO_3$; (*d*) that the transport of CO_2 is effected by the protein; and (*e*) that the alkali reserve of blood is constituted by (i) $NaHCO_3$ and (ii) protein.

(The expenses of this research have been defrayed by a grant from the Royal Society.)

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