CHAPTER IX

PRELIMINARY CONSIDERATIONS CONCERNING THE MODE OF COMBINATION OF CARBONIC ACID IN THE BLOOD

In the first seven chapters of this work the relations existing between the apparent hydrogen ion activity, the CO_2 tension and the combined carbonic acid of the blood have been developed. These relations depended on the assumption that all the combined carbonic acid in the various phases of the blood is present as bicarbonate[1], but on further scrutiny it will be obvious this proviso is by no means proved by the fact that the apparent hydrogen ion activity can be calculated from the modified Henderson-Hasselbalch equations. On the contrary it will be perceived that in the empirically determined "constants," corrections for combined carbonic acid not in the form of bicarbonate can very easily be hidden.

When I started these investigations I thought that the corroboration of Henderson's and Hasselbalch's "proof" that all the combined carbonic acid in the blood was present as bicarbonate, would not involve great theoretical difficulties so that by taking the heterogeneity of the solution into consideration it would be feasible to determine whether the evidence was really sound. But the more I have pondered over the theoretical conditions, the greater the difficulties have seemed to be. I have nevertheless, as far as I have been able, fulfilled my original plan, inasmuch as, it seems to me, questions of considerable interest have arisen but I have furthermore endeavoured to establish the proof in another way in order to avoid the most important difficulties. The evidence will be found in chapter XI but for the present it will be shown how far we can go with the original idea. The value that gives the greatest trouble is the apparent activity coefficient of the bicarbonate ion in the liquid phase of the blood corpuscles, and uncertainty in evaluating this will produce a significant error in the calculations in this chapter and chapter XII. I do not think however the conclusions arrived at can be disputed from a qualitative point of view but I quite realise that quantitatively, especially in this chapter, they may rather diverge from the results that may eventually be obtained.

In chapter III an equation was evolved for the calculation of the apparent hydrogen ion activity in the water phase of a two-phase system. With this equation (115) we can estimate the amount of bicarbonate which will be found in the serum proteins and haemoglobin if the combined carbonic acid is present as bicarbonate:

$$(115) \quad a_{H^\cdot} = \lambda_{(1)} \frac{P_{CO_2} a}{7{\cdot}60\,\beta},$$

[1] No account is taken here or henceforth of the small quantities of monocarbonate found in the blood (cf. chapter II).

the condition for which is

$$(117) \quad \lambda_{(1)} = K'_1 \frac{100 - q(1-d)}{100},$$

from which we obtain $$d = \frac{100\,(\lambda_{(1)} - K')}{K'_1 q} + 1, \quad \text{.....(151)}$$

in which the significance of the constants is discussed in chapters II and III. Let us first consider serum. We found in chapter V

$$\lambda_{(1)} = \lambda_{(s)} = 10^{-6 \cdot 294} = 5 \cdot 20 \times 10^{-7}.$$

K'_1 can be determined from the equation

$$(143) \quad -\log F_a(\mathrm{HCO'_3}) = 0 \cdot 46 \sqrt[3]{c}.$$

For the determination of the apparent activity coefficient we only lack the value of c.

Numerous and thorough investigations by Hamburger [1902], Hedin [1915], Ege [1920] and many others have shown that serum is almost isotonic (isosmotic) with a 0·9 % sodium chloride solution. For further information respecting this I would refer the reader to Rich. Ege's thesis. It would be erroneous however immediately to conclude that c was the same as in 0·9 % NaCl solution, that is $0 \cdot 154n$, because NaCl mols are not the only osmotically active ones. Now the mols which are not electrolytes are only present in such a small concentration in serum that for this purpose we can neglect them entirely. But at the reactions here in question there are also some protein anions present in serum which neutralise corresponding amounts of cations. The quantity of these never exceeds $0 \cdot 02n$ and presumably is usually about $0 \cdot 01n$, so we are justified in estimating c at between 0·16 and $0 \cdot 18n$.

The volume of the disperse phase in serum is certainly very nearly 8 % and putting this in equation (151) we obtain

$$c = 0 \cdot 16 \quad K'_1 = 10^{-6 \cdot 260} = 5 \cdot 50 \times 10^{-7} \quad d = 0 \cdot 32,$$
$$c = 0 \cdot 18 \quad K'_1 = 10^{-6 \cdot 251} = 5 \cdot 61 \times 10^{-7} \quad d = 0 \cdot 07.$$

The calculation is rather uncertain because a slight error in K'_1 or in $\lambda_{(s)}$ has a great effect, but it seems as though some bicarbonate is dissolved in the serum proteins.

Table XLIX. Calculation of the value of d in the blood corpuscles with equation (151).

		d	
c	$K'_1 \times 10^{-7}$	$q=40$	$q=35$
0·20	5·30	0·51	0·44
0·225	5·41	0·47	0·40
0·250	5·52	0·42	0·35

The concentration of cations in the water phase of the blood corpuscles is not certainly known. It has been estimated here as between 0·2 and 0·25. The reason for this will be discussed later in chapter XII.

$\lambda_{(1)} = \lambda_{(c)}$ in horse blood cell fluid, according to the measurements reported in chapter VII, is about 6·37. The volume of the disperse phase in blood cells

according to Ege's determinations is between 35 % and 40 %. If d is calculated with these values we obtain the results given in Table XLIX, using equation (58) with the constant 0·40, as a large part of the cations of blood cells consists of potassium.

From the table it will be seen that the value of d, under the given assumptions, lies between 0·35 and 0·51.

As emphasised above it is quite possible that the calculation may be rather uncertain quantitatively but there is hardly any doubt that d is really a positive quantity. If $d = 0$ and q and $\lambda_{(c)}$ at the same time have the values employed in the calculation above, we can estimate K'_1 and c since in that case the following holds:

$$K'_1 = \frac{100}{100 - q}\lambda_{(c)},$$

and therefore for $q = 40$ we get $K'_1 = 7{\cdot}12 \times 10^{-7}$ and $c = 0{\cdot}74n$, and for $q = 35$, $K'_1 = 6{\cdot}57 \times 10^{-7}$ and $c = 0{\cdot}55n$. These values for c are undoubtedly too high.

It may therefore be taken as settled that a part of the bicarbonate is dissolved in the haemoglobin provided that all the combined carbonic acid of the blood is really present as bicarbonate, and the calculations also show that the bicarbonate is about half as soluble in haemoglobin as in a roughly $n/4$ KCl solution (the dispersion medium).

This result is by no means antagonistic to what we already know about the solubility of salts in proteins. Thus S. P. L. Sörensen [1915–1917] found that ammonium sulphate was freely soluble in egg albumin but, on the other hand, it compels us to inquire whether a part of the combined carbonic acid is not present in some other form than the bicarbonate ion (or undissociated bicarbonate in the haemoglobin phase). It would be interesting to estimate first what amount of the combined carbonic acid of the blood may be expected to be dissolved in the protein phases according to the above calculations.

As an example let us take horse blood at 18° with a $p_{H'(s)}$ value of 7·40 and at a CO_2 tension of 40 mm., and further we will assume the volume of the blood cells is 40 % of the total volume of blood, which according to Fig. 10 corresponds to 19·6 vols. % combined O_2 (Haldane 106). We now calculate with (130) the combined carbonic acid (B) making the necessary correction with the help of Fig. 10, and we obtain 58·4 vols. %. From Fig. 7 we read that the partition ratio of combined CO_2 at this reaction (D) is 0·66.

In 100 cc. blood therefore there are 58·4 cc. combined CO_2 (0° and 760 mm.), of which 40·6 cc. are present in the 60 cc. serum, equivalent to a content of 67·6 cc. in 100 cc. serum; and in 40 cc. blood cells there are consequently 17·8 cc. combined CO_2 corresponding to 44·6 cc. in 100 cc. blood cells. Of the 60 cc. serum the water phase is 55·2 cc. and the protein phase (8 %) 4·8 cc., and if we assume d is 0·35, we obtain 39·4 in the water phase corresponding to 71·3 cc. in 100 cc. and 1·2 cc. in the protein phase which is equivalent to 25·0 cc. in 100 cc. protein. If we carry out a similar calculation for the blood corpuscles and put $q = 40$ and $d = 0{\cdot}45$, the water phase becomes 24 cc. and

the protein phase 16 cc. There are then 13·7 cc. in the water phase, equivalent to 57·2 cc. in 100 cc. and 4·1 cc. in the protein phase, equivalent to 25·7 cc. in 100 cc. In the two disperse phases there are altogether 4·1 + 1·2 = 5·3 cc. combined CO_2 which is equivalent to about 9 % of the total quantity of combined CO_2. We have found that 71·3 vols. % combined CO_2 are present in the water phase of serum, while there are only 57·2 vols. % in the water phase of the blood cells. Now the apparent activity coefficient of the bicarbonate ion is very nearly identical in the water phase of serum and blood cells because the greater cation concentration in the blood cells is almost balanced by the smaller Milner effect of the potassium ions when it is compared with the sodium ions, and the relation between the amounts of combined CO_2 in the two water phases will thus directly afford a measure of the relation between the activity of the bicarbonate ion in them, so that

$$\frac{a_{HCO_3} \text{ in the water phase of the blood cells}}{a_{HCO_3} \text{ in the water phase of the serum}} = 0{\cdot}8.$$

This result is by no means at variance with what we should expect, as it will be shown in chapter XII that on the basis of Ostwald's [1890] and Donnan's [1911] partition law such a distribution is to be looked for.

The above results are condensed in Table L.

Table L.

	Vols. % combined CO_2	Cc. combined CO_2
100 cc. blood at p_H· 7·40 and 40 mm.	58·4	58·4
In the serum of the above (60 cc.)	67·6	40·6
„ water phase of the serum (55·2 cc.) ...	71·3	39·4
„ protein phase of the serum (4·8 cc.) ...	25·0	1·2
„ blood cells (40 cc.)	44·6	17·8
„ water phase of the blood cells (24 cc.) ...	57·2	13·7
„ protein phase of the blood cells (16 cc.)	25·7	4·1

The amount of CO_2 combined in a form other than bicarbonate would thus appear to be small and, according to the above calculations, at most 9 % of the total. We will now try to get an idea of what form of combination this might theoretically be.

The combined carbonic acid is determined as the difference between the total dissociable combined CO_2 and the dissolved amount calculated by Henry's law. The relative absorption coefficient of blood and serum has been determined indirectly by Bohr [1905], the assumption being that it is the same for all gases. This is of course only approximately true but the divergence is not so large that a grave error can arise if the CO_2 tension is not too high. It is however possible that CO_2 could be adsorbed at the interface between protein and the dispersion medium. That such an adsorption actually takes place in blood is claimed by Findlay for various gases as will be referred to in the next chapter but he undoubtedly overestimates the significance of his experiments. It must also be noted that $\lambda_{(c)}$ could not be independent of the reaction if such an adsorption took place for reasons identical with those that will be advanced at the end of this chapter against the existence of a protein-carbamino-acid.

The possibility may also be raised that some of the bicarbonate may be present as undissociated protein bicarbonate (complex salt). This assumption is however not quite correct because in alkaline reactions only very small or negligible amounts of protein cations are present, since proteins function almost entirely as acids and so there can be no question here of undissociated protein bicarbonate. At reactions in the region of, or more acid than, $10^{-7 \cdot 0}$ some haemoglobin cations are in all probability formed so that there is the possibility of the existence of undissociated haemoglobin bicarbonate and in such a case $p\lambda_{(c)}$ might decrease with increasing hydrogen ion activity, but in chapter VII we saw this was not so. We can therefore conclude that undissociated bicarbonate does not exist in sufficient quantity to compromise our theories and calculations. Further it is possible that the bicarbonate ion might be adsorbed at the interface between protein and salt solution. As a matter of experience however such an adsorption only takes place when the disperse phase has a charge of opposite sign to that of the adsorbed ions, and so the theory of the adsorption of the bicarbonate ion by proteins is untenable for the same reason as applies to undissociated protein bicarbonate. Furthermore the problem of the adsorption of the ions is presumably identical with the problem of the large Milner effect of the colloid ions, according to the views of which Prof. Bjerrum has kindly given me the benefit. There remains the possibility that some of the CO_2 combines with the protein in some other form than protein bicarbonate. Such a form of combination has been stated by Siegfried to be protein carbamino-acid.

It will be clear to the reader that the combined carbonic acid referred to in this work is only the dissociable combined carbonic acid, that is to say the carbonic acid which can be liberated by the addition of acid or by diminishing the CO_2 tension; that which can only be split off from the organic molecules by such vigorous processes that the latter are destroyed is naturally not included, it can moreover be shown that this dissociation of the carbonic acid combination of the blood is completely reversible. If protein carbamino-acid took part in the reversible carbonic acid combination $\lambda_{(c)}$ (or $\lambda_{(m)}$) could not be constant in the fluid of the blood cells because the non-ionised combined H_2CO_3 would then vary with the CO_2 tension. This evidence that all the combined carbonic acid is really ionised in the blood is well supported by the determinations of $p\lambda_{(m)}$ in chapter VII, but is weakened to some extent by the determinations being so extremely difficult to carry out. But it also involves the assumption that q and d in equation (117) do not vary appreciably with the hydrogen ion activity. From a close examination of (117) however it appears the variations must be fairly large to make themselves felt in the determinations in question.

As it would certainly involve very great experimental difficulties to proceed further with the questions raised in this chapter there is every inducement to examine the literature for experiments which can throw light on these problems.

In conclusion it may be remarked that the experiments and views put forward give no information as to whether the bicarbonate dissolved in the protein phase is present exclusively as ions, or as undissociated salt as well.

RÉSUMÉ.

It has been shown that a small part of the combined carbonic acid is not present in the dispersion medium of the serum and blood cells, and the manner of combination of this amount of combined acid is discussed. It is probable that it is dissolved in the protein phases.

CHAPTER X

A BRIEF HISTORICAL REVIEW OF THE OLDER THEORIES OF THE COMBINATION OF CO_2 IN THE BLOOD.

The first to extract gas out of blood was John Mayow of Oxford (quoted from P. Bert), who reported his results in a paper in 1674. He thought the gas obtained was what we now call oxygen.

In 1783 Pietro Moscati of Milan stated he had extracted a gas out of blood and he believed he had proved it to be carbonic acid (fixed air), but his evidence was not good as by allowing blood to stand for 24 hours with lime water he obtained a precipitate [1784].

In 1799 Humphrey Davy proved that CO_2 could be driven out of blood by heating it, and in 1814 Vogel showed that CO_2 could be pumped out of blood. Up to 1837 when Magnus finally established that CO_2 could be pumped out of blood, the question was one of considerable interest as it was closely bound up with the current discussion on where oxidation took place in the organism.

Lavoisier had originally discussed whether oxidation occurred in the peripheral circulation or in the lungs. In his later works he claimed to have proved it took place in the lungs (according to W. Edwards and Ch. J. B. Williams). He believed that the carbon in a state of fine division was carried by the blood to the lungs and burned up there in association with oxygen. It became therefore of prime importance at that time to ascertain whether carbonic acid (CO_2) was free in the blood or only in a state of combination. In those days it was evidently not considered sufficient that carbonic acid could be driven out of the blood by heat, or by treatment with another gas such as hydrogen or atmospheric air, because it was supposed these gases replaced carbonic acid in loose combinations although it was partly realised similar processes took place in the lungs.