

RÉSUMÉ.

A search of the old literature does not disclose any facts which would lead us to abandon Zuntz's theory modified in accordance with modern physico-chemical requirements.

CHAPTER XI

FURTHER CONSIDERATIONS AND EXPERIMENTS ON THE NATURE OF THE COMBINATION OF CO₂ IN THE BLOOD, ILLUSTRATED BY THE "CARBONIC ACID COMBINATION" CURVE¹.

In a solution in equilibrium with air containing CO₂, a quantity of CO₂ is found to be dissolved according to Henry's law, as repeatedly stated. This CO₂ can be extracted from the solution by diminishing the CO₂ tension in the gas phase over the solution, and further by this process more CO₂ which must have been reversibly "combined" in the solution can often be liberated.

It has frequently been stated in earlier chapters that the combined CO₂ may be present

- (1) as adsorbed CO₂ if the solution is heterogeneous,
- (2) as carbonate ions (monocarbonate and bicarbonate),
- (3) as undissociated carbonate (complex salt),
- (4) as adsorbed carbonate which will presumably be identical with (3), since the molecular groups lying on the surface in a high molecular solute may be expected under certain circumstances to form compounds with the carbonate,
- (5) as a dissociable combination in which CO₂ takes part after assumption into the molecule, *e.g.* carbamino-acids.

At reactions more acid than $a_{\text{H}^+} = 10^{-8}$ we can regard the carbonate ions present solely as bicarbonate (cf. chapter II).

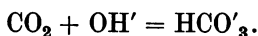
We will first examine systems in which adsorption combinations and complex salts are not found. In general, the following equation for the equilibrium of the positive and negative charges is valid for any solution whatever:

$$C_{M^+} + 2C_{M^{++}} + 3C_{M^{+++}} + \dots nC_{M^{+n}} \dots n \text{ charges} \\ = C_{A^-} + 2C_{A^{--}} + 3C_{A^{---}} + \dots nC_{A^{-n}} \dots n \text{ charges}, \dots (152)$$

where C_{M^+} stands for the concentration of monovalent cations and C_{A^-} for the concentration of monovalent anions and so on. As hydrogen and hydroxyl ions at reactions not far removed from the neutral point only occur in very small concentrations we can neglect them as terms in the above equation.

¹ D. D. van Slyke [1921, 1] has recently reviewed the question of the combination of CO₂ in blood from the theoretical as well as from the clinical standpoint. Those interested are referred to the original articles which only became known to me after the following had been written.

In a solution in which only strong acids and their salts are present combined CO_2 cannot exist as there are no cations to counterbalance the bicarbonate ions. But in a solution containing a free base the hydroxyl ions will react thus:



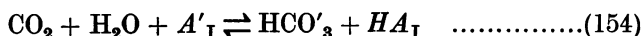
Under the given conditions this reaction practically proceeds quantitatively. In such a solution the combination of CO_2 will, as is well known, be independent of the pressure (if monocarbonate is not formed).

If a weak base or the salt of a weak acid is present in a solution, CO_2 will be combined in amounts varying with the CO_2 pressure if the reaction is suitable.

For a weak monovalent acid, as shown in chapter I, the following equation is valid:

$$\frac{a_{\text{H}'} \times a_{\text{A}'_1}}{a_{\text{HA}'_1}} = K_{\text{HA}'_1} \dots\dots\dots(153)$$

On saturation the following reaction



proceeds in such a way that a part of A'_1 is substituted by HCO'_3 until the equilibrium

$$K_1 \frac{a_{\text{A}'_1}}{a_{\text{HA}'_1}} = \frac{a_{\text{HCO}'_3}}{a_{\text{H}_2\text{CO}_3}} K_{\text{HA}'_1} \dots\dots\dots(155)$$

is reached.

If a weak monovalent base is present in a solution the reaction



proceeds until the following equilibrium is reached:

$$\frac{a_{\text{M}'_1}}{a_{\text{M}'_1\text{OH}}} \times \frac{K_W}{K_{\text{M}'_1\text{OH}}} = K_1 \frac{a_{\text{HCO}'_3}}{a_{\text{H}_2\text{CO}_3}} \dots\dots\dots(157)$$

The base is thus dissociated with the production of cations and bicarbonate ions. When equilibrium is attained an amount of bicarbonate ions will be present in the solution equivalent either to the cations the dissociation of which does not vary with the reaction (which before equilibrium were balanced by other anions the dissociation of which varies with the reaction) or to newly formed cations the dissociation of which varies with the reaction. The amount of combined CO_2 therefore becomes a measure of the *available cation concentration*. D. D. van Slyke and his collaborators have used the term "alkali reserve" for this amount (in serum) and E. Jarlöv has employed the almost analogous expression "available alkali." The reason I consider these expressions should be given up is because of the possibility of misunderstanding them. Thus Parsons, Davies, Haldane and Kennaway have erroneously assumed that in the "maximum" amount of CO_2 combined we have a measure of the difference between the cations and anions the dissociation of which does not vary with the reaction.

In order to illustrate the above I have performed experiments with sodium hydroxide solutions to which NaCl and phosphate solutions were added. The experiments will be found in Table LI and graphically in Fig. 15.

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Table LI.

mm. Hg CO ₂ 1	Vols % CO ₂ combined 2	pH calc. 3	$a_{H^+} \times 10^{-7}$ calc. 4	With equation (171)		pH calc. 7	With equation (172)		With equation (180)	
				Vols % CO ₂ combined 5	Diff. Vols. % 6 (2-5)		Vols % CO ₂ combined 8	Diff. Vols. % 9 (2-8)	Vols % CO ₂ combined 10	Diff. Vols. % 11 (2-10)
I. 30 cc. <i>m</i> /15 KH ₂ PO ₄ + 50 cc. 0.04941 <i>n</i> NaOH + H ₂ O to 100 cc. + 0.42 g. NaCl. 20°.										
7.0	16.1	7.585	0.260	17.0	-0.9	7.608	17.6	-1.5	15.9	+0.2
12.7	19.4	7.407	0.385	19.9	-0.5	7.418	20.2	-0.8	19.7	-0.3
23.4	23.5	7.225	0.596	23.7	-0.2	7.229	23.8	-0.3	23.6	-0.1
46.4	28.0	7.004	0.990	28.7	-0.7	7.015	29.1	-0.9	28.3	-0.3
76.5	31.9	6.844	1.43	32.7	-0.8	6.854	33.2	-1.3	32.4	-0.5
172.3	38.4	6.571	2.68	39.3	-0.9	6.582	39.8	-1.4	37.6	+0.8
745.0	48.7	6.038	9.15	48.8	-0.1	6.039	49.2	-0.5	49.1	-0.4
II. 6 cc. <i>m</i> /15 Na ₂ HPO ₄ + 24 cc. <i>m</i> /15 KH ₂ PO ₄ + 50 cc. 0.04941 <i>n</i> NaOH + H ₂ O to 100 cc. + 0.42 g. NaCl. 19°.										
8.6	49.2	7.954	0.111	49.4	-0.2	7.956	49.4	-0.2	43.1	+6.1
14.4	51.0	7.746	0.180	51.2	-0.2	7.747	51.3	-0.3	47.8	+3.2
20.2	52.1	7.608	0.247	52.9	-0.8	7.614	52.8	-0.7	50.7	+1.4
26.7	53.5	7.498	0.318	54.3	-0.8	7.504	54.6	-1.1	53.2	+0.3
47.3	57.7	7.283	0.521	58.4	-0.7	7.288	58.6	-0.9	57.9	-0.2
82.5	62.0	7.073	0.846	63.0	-1.0	7.080	63.0	-1.0	62.7	-0.7
436.4	77.5	6.446	3.58	78.8	-1.3	6.453	78.1	-0.6	76.4	+1.1
750.2	80.6	6.228	5.92	82.9	-2.3	6.240	82.1	-1.5	81.4	-0.8
III. 30 cc. <i>m</i> /15 Na ₂ HPO ₄ + 50 cc. 0.04941 <i>n</i> NaOH + H ₂ O to 100 cc. + 0.42 g. NaCl. 20°.										
5.2	56.3	8.240	0.0576	56.7	-0.4	8.242	56.8	-0.5	48.3	+8.0
12.5	58.8	7.878	0.132	58.8	0.0	7.878	58.8	0.0	54.1	+4.7
19.6	60.4	7.694	0.202	60.7	-0.3	7.696	60.8	-0.4	58.1	+2.3
25.2	62.4	7.599	0.252	61.8	+0.6	7.595	61.9	+0.5	60.2	+2.2
43.5	65.4	7.383	0.415	65.4	0.0	7.383	65.4	0.0	64.9	+0.5
81.0	70.1	7.142	0.721	70.3	-0.2	7.144	70.4	-0.3	70.1	0.0
186.2	77.4	6.824	1.50	78.1	-0.7	6.828	78.1	-0.7	77.0	+0.4
414.4	84.2	6.513	3.07	85.5	-1.3	6.520	85.7	-1.5	83.8	+0.4
731.5	87.5	6.283	5.21	90.1	-2.6	6.296	90.2	-2.7	88.8	-1.3
IV. 24 cc. <i>m</i> /15 Na ₂ HPO ₄ + 6 cc. <i>m</i> /15 KH ₂ PO ₄ + 0.0243 <i>n</i> Na ₂ CO ₃ to 100 cc. + 0.85 g. NaCl. The two first experiments at 18°, the others at 19°.										
6.9	32.8	7.835	0.146	33.3	-0.5	7.841	33.4	-0.6	27.6	+5.2
10.4	34.1	7.673	0.212	34.9	-0.8	7.683	35.0	-0.9	31.4	+2.7
21.1	37.6	7.416	0.384	38.6	-1.0	7.427	38.7	-1.1	37.3	+0.3
42.7	42.4	7.162	0.689	43.6	-1.2	7.174	43.9	-1.5	43.1	-0.7
85.3	48.6	6.921	1.20	49.3	-0.7	6.927	49.5	-0.9	48.7	-0.1
221.8	57.1	6.575	2.66	58.0	-0.9	6.582	58.3	-1.2	56.7	+0.4
414.0	63.0	6.347	4.50	63.0	0.0	6.347	63.0	0.0	62.0	+1.0
747.3	67.0	6.117	7.63	66.8	+0.2	6.117	66.6	+0.4	67.3	-0.3
747.3	66.6	6.115	7.68	66.8	-0.2	6.114	66.8	-0.2	67.3	-0.7
V. 24 cc. <i>m</i> /15 Na ₂ HPO ₄ + 6 cc. <i>m</i> /15 KH ₂ PO ₄ + 50 cc. 0.04941 <i>n</i> NaOH + H ₂ O to 100 cc. + 0.85 g. NaCl. 19°.										
10.0	49.9	7.859	0.138	50.1	-0.2	7.861	50.1	-0.2	47.4	+2.5
22.7	53.0	7.529	0.296	53.7	-0.7	7.534	53.9	-0.9	53.8	-0.8
47.7	57.8	7.244	0.570	58.9	-1.1	7.252	59.1	-1.3	59.3	-1.5
85.2	62.3	7.023	0.945	63.9	-1.6	7.036	64.1	-1.8	63.6	-1.3
178.6	68.5	6.744	1.80	70.9	-2.4	6.759	71.2	-2.7	69.0	-0.5
375.2	76.9	6.473	3.37	77.6	-0.7	6.476	77.7	-0.8	74.2	+2.7
743.2	81.0	6.198	6.34	82.6	-1.6	6.207	82.6	-1.6	79.5	+1.5

In the first three solutions 0.42 % NaCl was present and 50 cc. 0.04941*n* NaOH in 100 cc. solution. In the last two solutions 0.85 % NaCl was present and in the first of this series 70 cc. 0.0243*n* Na₂CO₃ in 100 cc. In the last, NaOH was of the same concentration as in the first three. In all the solutions there was also 0.02*m* phosphate (30 cc. of S. P. L. Sørensen's phosphate mixtures to 100 cc. solution), the alkali content of the solutions being varied by adding different amounts of primary and secondary phosphate. The experiments were performed as usual (with the small saturator). In the first column of the table the CO₂ tension is given; in the second column the combined CO₂ in vols. %; in the third column *p*_H calculated in the usual way, the values for *K*'₁ given later being used; the fourth column contains *α*_H.

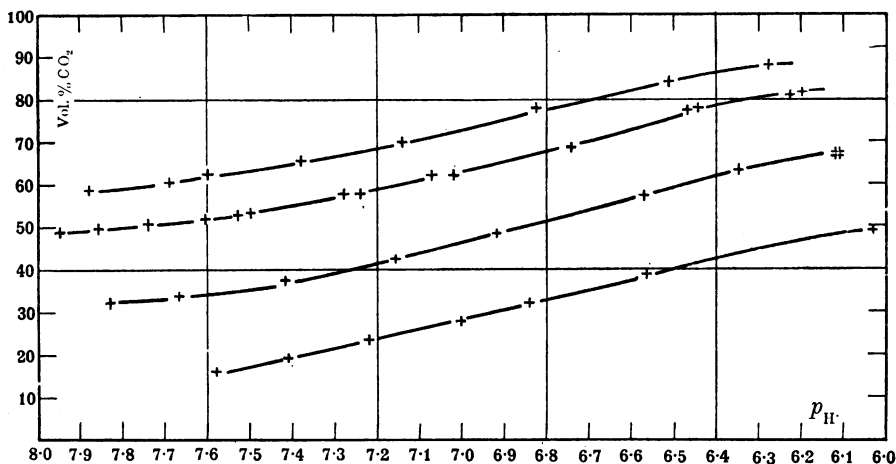
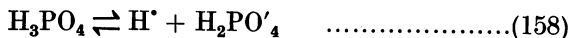


Fig. 15. The combination of CO₂ in 0.02*m* phosphate solution.

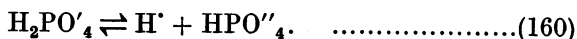
We will let *C*_I stand for the difference between the total cations and total anions the dissociation of which does not depend upon the reaction. The value for *K*'₁ employed is calculated by equations (139) and (143). The first dissociation of phosphoric acid



progresses, under the conditions of the experiment, from left to right completely, the dissociation constant according to Abbott and Bray [1909] being 10^{-2.0}. The third dissociation of phosphoric acid under the same conditions does not take place at all, that is to say the reaction



proceeds completely from right to left, the dissociation constant according to Abbott and Bray being 10^{-12.5}. The only significant reaction therefore is



At a given CO₂ tension the following equilibrium holds for (160):

$$a_{\text{H}^+} = K'_1 \frac{P_{\text{CO}_2} \alpha}{7.60B} = k_2 \frac{C_{\text{H}_2\text{PO}'_4} F_a(\text{H}_2\text{PO}'_4)}{C_{\text{HPO}''_4} F_a(\text{HPO}''_4)} \dots\dots\dots(161)$$

(An almost analogous equation was evolved by L. J. Henderson as early as 1908.)

If we now put $k_2 \frac{F_a(\text{H}_2\text{PO}'_4)}{F_a(\text{HPO}''_4)} = k'_2 \dots\dots\dots(162)$

we get $a_{\text{H}^+} = K'_1 \frac{P_{\text{CO}_2} \alpha}{7.60B} = k'_2 \frac{C_{\text{H}_2\text{PO}'_4}}{C_{\text{HPO}''_4}} \dots\dots\dots(163)$

The total phosphate concentration is expressed by

$$C_{\text{Ph}} = C_{\text{H}_2\text{PO}'_4} + C_{\text{HPO}''_4} \dots\dots\dots(164)$$

The charge expressed in normalities corresponding to the phosphates then becomes

$$uC_{\text{Ph}} = C_{\text{H}_2\text{PO}'_4} + 2C_{\text{HPO}''_4} \dots\dots\dots(165)$$

From (163), (164) and (165) we get

$$u = \frac{15.20Bk'_2 + K'_1 P_{\text{CO}_2} \alpha}{7.60Bk_2 + K'_1 P_{\text{CO}_2} \alpha} \dots\dots\dots(166)$$

or $u = \frac{2k'_2 + a_{\text{H}^+}}{k'_2 + a_{\text{H}^+}} \dots\dots\dots(167)$

Further, C_I is defined by

$$C_I = C_{\text{HCO}'_3} + C_{\text{H}_2\text{PO}'_4} + 2C_{\text{HPO}''_4} \dots\dots\dots(168)$$

and therefore from (168) and (165)

$$B = (C_I - uC_{\text{Ph}}) 2226. \dots\dots\dots(169)$$

From (169) and (166) we obtain

$$B^2 + B \frac{K'_1 P_{\text{CO}_2} \alpha + 2226 \times 7.60k'_2 (2C_{\text{Ph}} - C_I)}{7.60k_2} + \frac{K'_1 2226 P_{\text{CO}_2} \alpha (C_{\text{Ph}} - C_I)}{7.60k'_2} = 0. \dots\dots(170)$$

Substituting in this the constants given in Table LI a we get, for the first series $B = 5.2 - 0.217 P_{\text{CO}_2} + \sqrt{0.0471 P^2_{\text{CO}_2} + 21.5 P_{\text{CO}_2} + 27.0}$;

for the second series

$$B = 23.0 - 0.230 P_{\text{CO}_2} + \sqrt{0.0529 P^2_{\text{CO}_2} + 31.6 P_{\text{CO}_2} + 529}$$

for the third series

$$B = 27.5 - 0.226 P_{\text{CO}_2} + \sqrt{0.0511 P^2_{\text{CO}_2} + 32.6 P_{\text{CO}_2} + 756}$$

for the fourth series at 18°

$$B = 14.5 + 0.252 P_{\text{CO}_2} + \sqrt{0.0635 P^2_{\text{CO}_2} + 29.8 P_{\text{CO}_2} + 210}$$

at 19° $B = 14.5 + 0.248 P_{\text{CO}_2} + \sqrt{0.0615 P^2_{\text{CO}_2} + 29.3 P_{\text{CO}_2} + 210}$;

for the fifth series

$$B = 23.0 + 0.250 P_{\text{CO}_2} + \sqrt{0.0626 P^2_{\text{CO}_2} + 33.9 P_{\text{CO}_2} + 529} \dots\dots\dots(171)$$

Table LI a. Constants used in the calculation of (171).

Series	Total cation concentration	C _I	K' ₁	C _{Ph}	k' ₂	Temp.
I	0.1165	0.0447	10 ^{-6.286} = 5.18 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	19
II	0.1325	0.0607	10 ^{-6.278} = 5.36 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	20
III	0.1365	0.0647	10 ^{-6.266} = 5.40 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	19
IV (18°)	0.1940	0.0530	10 ^{-6.244} = 5.70 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	18
IV (19°)	0.1940	0.0530	10 ^{-6.239} = 5.77 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	19
V	0.2027	0.0607	10 ^{-6.235} = 5.82 × 10 ⁻⁷	0.02	10 ^{-6.86} = 1.38 × 10 ⁻⁷	19

k'_2 is taken as $10^{-6.86}$, which has been very accurately determined by S. P. L. Sørensen [1909-1910] in a mixture of equal parts of $m/15$ KH_2PO_4 and $m/15$ Na_2HPO_4 . It is probable that the constant in our case only differs slightly from this value. In solutions of 0.42 % NaCl , Y is taken as 6, in other solutions, as 8. The results of the calculations will be found in Table LI in the fifth column; in the sixth column the differences between the values found and calculated are given. If we put aside the experiments with the highest CO_2 tensions (which are subject to the largest experimental error), it will be seen the agreement is as good as could be expected on the whole, but it should be noted the calculated value is always a little greater than that found by experiment. The cause of this is not quite clear but it may be pointed out that the agreement would have been very nearly ideal if k'_2 was taken as $10^{-6.80}$.

Now that we have effected these calculations let us turn again to the equations (171). It will immediately be obvious that it is difficult to form a clear conception of the course of the five carbonic acid combination curves if we only have these equations to go by, but it will be simple if from (167) and (169) we evolve the equation

$$B = (C_I - C_{\text{Ph}} \frac{2k'_2 + a_{\text{H}^+}}{k_2 + a_{\text{H}^+}}) 2226. \quad \dots\dots\dots(172)$$

This equation indicates that all the CO_2 combination curves in solutions which contain the same phosphate concentration run parallel with one another if they are drawn in a right angled coordinate system where the apparent hydrogen ion activity, or a value which contains no other variable than this, is placed along one axis and the combined CO_2 along the other. If the combined CO_2 is expressed in vols. % the distance between the two curves projected on the vols. % axis will be the difference between the $C_I \times 2226$ (vols. %) of the two solutions.

The above reactions are of general interest in that, in any similar solution whatever we have

$$B = (C_I - C_V f(a_{\text{H}^+})) 2226, \quad \dots\dots\dots(173)$$

where C_V is the concentration of the electrolytes of which the dissociation varies with the reaction, and where C_I and $C_V f(a_{\text{H}^+})$ can have positive or negative values. Even though we do not know C_I , C_V , or $f(a_{\text{H}^+})$ we can, under the given assumptions, be certain of the shift in the CO_2 combination curve if C_I varies on the addition of alkali (or acid), the volume of the system being constant. If for instance C_I is increased to $C_I + b$ we get

$$B_I = (C_I + b - C_V f(a_{\text{H}^+})) 2226. \quad \dots\dots\dots(174)$$

Subtracting (173) from this we obtain

$$B_I - B = 2226b, \quad \dots\dots\dots(175)$$

which is the algebraic expression of the fact that any CO_2 combination curve will be shifted parallel with itself in an a_{H^+} -vols. % CO_2 graph provided adsorption combinations of CO_2 , or CO_2 compounds in the form of complex

bodies (*e.g.* carbamino-acids), are not formed. The distance between the curves is then $2226b$ vols. %.

If the CO_2 in a solution can be adsorbed or combined in the complex manner indicated above and if these combinations are reversible, the amount combined will be a function of the CO_2 pressure. The general expression for the combination will be

$$B = (C_I - C_V f(a_{\text{H}^+}) - f_1(P_{\text{CO}_2})) 2226, \dots\dots\dots(176)$$

and the difference in CO_2 combined in a solution before and after the addition of alkali at the same reaction will therefore, from analogy with (175), be

$$B_I - B = 2226b - 2226 (f_1(P_{\text{CO}_2}) - f_1(P_{\text{CO}_2'})). \dots\dots\dots(177)$$

In this equation the amount

$$- 2226 (f_1(P_{\text{CO}_2}) - f_1(P_{\text{CO}_2'}))$$

will always be positive because the combination of CO_2 must increase with the CO_2 tension in the given conditions and in order to produce the same reaction a higher CO_2 tension will be necessary after the addition of alkali than before (*cf.* equation (95)).

It is thus possible to state in general that a CO_2 combination curve, in which the combined CO_2 is set off along the one axis and an expression for the active reaction along the other axis, will be shifted on the addition of alkali in such a way:

(1) that the distance between the original curve and the new curve is always equal to the combination of CO_2 corresponding to the added amount of alkali provided that the variable combination in each curve is only a function of the reaction;

(2) that the distance between the original and new curves will increase with the apparent hydrogen ion activity provided that the variable combination is also a function of the CO_2 tension;

(3) that the distance between the two curves can never be less than the combined CO_2 corresponding to the added alkali.

We will now revert to the phosphate solutions again. In the eighth column of the table the combined CO_2 expressed in vols. % will be found, calculated by (172) with the help of the apparent hydrogen ion activity in column 4 reckoned directly from the experiments. The agreement is, as expected, good. In Fig. 15 the combined CO_2 is plotted as ordinates and

$$- \log a_{\text{H}^+} = p_{\text{H}^+}$$

as abscissae, however on a hundred times greater scale. In such a coordinate system, as explained, the course of the curves will be parallel. Now it appears from the figure that the CO_2 combination curves are straight lines for a long portion of their courses, and we will therefore, as an example of what is to follow, calculate these sections of the curves by the method of least squares, using the figures in columns 2 and 3, and include all experiments in which

p_{H^+} is less than 7.59. Equations (141) and (142) are employed in the calculation and the mean error of the constants is estimated by the equation

$$M_x = M_y = \frac{M}{\sqrt{n(\Sigma a^2 - (\Sigma a)^2)}}, \dots\dots\dots(178)$$

where

$$M = \sqrt{\frac{\Sigma a^2}{n-2}}, \dots\dots\dots(179)$$

Σa being the sum of the squares of the deviations of the found and calculated values and n the number of experiments. The equation of the straight line referred to above is

$$x \log a_{\text{H}^+} + y = B, \dots\dots\dots(180)$$

where x is the tangent to the angle made by the line with the axis of the abscissa, measured in the first or third quadrant, and y is the part of the ordinate axis cut off by the line. The calculation gives

Series	x	y	M	No. of experiments
I	21.460 ± 0.057	178.65 ± 0.057	0.51	7
II	22.139 ± 0.17	219.15 ± 0.17	0.91	5
III	21.720 ± 0.16	225.24 ± 0.16	0.87	5
IV	23.076 ± 0.077	208.41 ± 0.077	0.68	7
V	19.301 ± 0.27	199.13 ± 0.27	1.9	6

It will be seen that a determination of the error indicates that x is different in the various series, but as it has been proved already by calculations that the different curves are really parallel, allowing for experimental error, we cannot attribute any real significance to the mean error calculated by this method in the present and similar cases. There are several reasons for this rather unsatisfactory result. In the first place the number of experiments in each series, within the range of reaction employed, is far too few to permit of an accurate determination of the error; in the second place the points determined are not regularly distributed over the various sections of the curve, in fact they do not all cover exactly the same range of reaction, which is of importance as it will be shown the portion of the curve in question is not in reality a straight line. For the appreciation of what is to follow it will be necessary to examine this a little more closely.

By equation (172) it is easy to estimate the true slope of the curve. In the second column of the following table the combinations of CO_2 in a 0.02*n* phosphate solution of p_{H^+} varying from 7.50 to 6.20 are given, 2226 $C_{\text{I}} = 52.3$ vols. %, the combined CO_2 at p_{H^+} 7.50 being here put equal to 0. In the third column will be found the tangent to the straight lines (angle of inclination which is x) which join the nearest points together two by two. If the point referring to p_{H^+} 7.50 is joined with that referring to p_{H^+} 6.20 the tangent to the angle of inclination of the line is 21.6. In the fourth column will be found the amount of combined CO_2 expressed in vols. %, which, according to a calculation based on the straight line smoothing formulae (141) and (142) from the figures in columns 1 and 3, should be found combined at the given p_{H^+} when the best constants are used. These constants are

$$x = 22.719 \pm 0.021, \quad y = 169.77 \pm 0.021.$$

In the fifth column the differences between the CO_2 combinations calculated by (172) and by the smoothing are given. The standard deviation is calculated to 0.45 vols. %.

Table LI b.

pH	Vols. % CO ₂ combined. Calculated by (172)	x	Vols. % CO ₂ combined. Algebraic smoothing	Difference vols. % 5 (2-4)
1	2	3	4	5
7.50	0.0		-0.6	+0.6
7.40	1.7	17	1.7	0.0
7.30	3.6	19	3.9	-0.3
7.20	5.7	21	6.2	-0.5
7.10	8.0	23	8.5	-0.5
7.00	10.4	24	10.7	-0.3
6.90	12.9	25	13.0	-0.1
6.80	15.5	26	15.3	+0.2
6.70	18.0	25	17.6	+0.4
6.60	20.4	24	19.8	+0.6
6.50	22.6	22	22.1	+0.5
6.40	24.7	21	24.4	+0.3
6.30	26.5	18	26.6	-0.1
6.20	28.1	16	28.9	-0.8

If column 5 is examined it will be observed that the differences change their sign three times, that is to say the straight line cuts the true curve (calculated by (172)) three times. If it should happen, in the determination of a similar CO₂ combination curve, that points were obtained at these places of intersection, it would naturally follow that even an ideal method of analysis would be unable to show that the curve departed from a straight line. It will be realised that a very considerable number of experimental points are required to determine the true course of the curve and that in using the smoothing method of calculation one must be exceedingly cautious about one's conclusions.

As mentioned the constant x was determined by the smoothing method to be 22.719 ± 0.021 . If this is compared with the constants found by calculation from the experiments it will be seen that only that from series II appears to be of the same magnitude, which is another plea for the contention that the mean error of the constants is really too sharply defined.

There is still another point in relation to the smoothing method to be touched upon. It is not possible by this method in the form which is used here with the aid of the mean error of the constants to determine the mean error from a single point on the curve. This is due to the choice of the zero point of the coordinate system, because this lies, as will be seen, far outside the experimentally determined points. The reason I have given the numbers for the constants correct to the first decimal place in spite of the objections

put forward relating to the mean error, is in order to show them with all the figures used in the calculations and the mean error is therefore only employed roughly to demonstrate the uncertainty of the constants.

It is accepted that a large part of the reversibly combined CO_2 of the blood is present as the bicarbonate ion, the haemoglobin and other protein substances acting as ampholytes. The objections against this view which have been put forward in the last few years have been to some extent negated in previous chapters and will also be questioned at the end of the present one. It can therefore be concluded that no general condition for the combination of CO_2 in the blood can be formulated which cannot be traced back to an equation of the form of (173) or (176). Provided no CO_2 is adsorbed or combined in a complex manner (173) will be the correct equation.

In 1920 T. R. Parsons put forward a mathematical treatment of the CO_2 combination in the blood but it seems to me he has made a mistake on this point. Firstly Parsons assumed that haemoglobin is a monobasic acid, each haemoglobin molecule however encompassing several such groups. Secondly he assumed that in blood especially there were so many more cations than anions the dissociation of which did not vary with the reaction, that they were able just to neutralise the haemoglobin acid. Thirdly he believed that in the "maximum" of combined CO_2 we have a measure of the concentration of the haemoglobin acid.

The first of these assumptions is quite uncertain; the second is undoubtedly erroneous, because the blood can expel CO_2 from alkaline carbonate as Pflüger [1864] was the first to show. The third assumption is incorrect as there is no maximal CO_2 combination in blood at atmospheric CO_2 tensions. Parsons' final relation evolved as it is from faulty assumptions has no real significance and it is only one of many instances that nearly every equation with a sufficient number of constants can be brought into agreement with an observed fact when the constants chosen are the best possible.

At present there seems to be no possibility—contrary to what was the case with the phosphate experiments—of establishing an *a priori* relation between α_{H^+} and the combination of CO_2 with the blood proteins. There is no other course open therefore than to find the simplest empirical relation between these values.

In the previously mentioned paper of Hasselbalch and Warburg some CO_2 combination curves for blood and serum are given in a p_{H^+} —vols. % curve. They are straight lines. This fact appears to have been quite overlooked, but I believe the appreciation of this phenomenon will be of far-reaching importance in the physiology of respiration. In this work I shall content myself with more thoroughly demonstrating the relation and employ it for elucidating the question of the existence of complex CO_2 combinations as well as some other problems.

In tables LII—LVII (pp. 287—290) the combination of CO_2 at various CO_2 tensions in a number of blood samples from different people and animals is given. In the last column but one the results of the algebraic smoothing are recorded, the CO_2 combination curve in the p_{H^+} —vols. % combined CO_2 chart being taken as a straight line.

It is therefore assumed that

$$x \log a_{\text{H}^+} + y = B \quad \dots\dots\dots(181)$$

or

$$- x p_{\text{H}^+} + y = B. \quad \dots\dots\dots(182)$$

That these relations, within the experimental error, are really true seems to follow from the curves and tables, but I have further investigated some rather extensive unpublished material dealing with the CO₂ combination curves in ox blood which has been elaborated of late years for another purpose in the laboratory of the Finsen Institute, without being able to find any sign of a departure from the relations. After what was said about the phosphate curves it will readily be understood I do not wish to urge that the sections of the curves being dealt with are actually straight lines, but it is asserted the deviations from a straight line are small compared with the variations in the combination of CO₂ at different apparent hydrogen ion activities.

In the calculation of $p_{\text{H}^+(s)}$ (130) was used. In the case of oxygenated human blood at 38° and oxygenated horse blood at room temperature the corrections given in Fig. 10 were added to the values found. In the case of reduced human blood at 38° and reduced horse blood at room temperature the correction corresponding to p_{H^+} (uncorrected - 0.40) was added. In the case of Hasselbalch's [1916] "half reduced" blood 0.20 was subtracted from p_{H^+} (uncorrected), while 0.40 was subtracted from oxygenated ox blood at room temperature. Equation (130) was used without correction in the calculation of the other blood samples.

The correction curve corresponding to 18.5 vols. % combined CO₂ was used in the calculations relating to Haldane's blood, from Christiansen, Douglas and Haldane [1914], the 15 vols. % curve for Parsons' [1917] blood, the 20 vols. % curve for Davies' [1920] and Hasselbalch's [1916, 2] blood and the 25 vols. % curve for Joffe's [1920] and Warburg's blood¹. In the calculations

¹ I have not attempted to discover how much combined O₂ was actually present in Davies', Haldane's, Joffe's and Parsons' blood but I have contented myself with estimating their p_{H^+} -CO₂ combination curves by means of the corrections mentioned because an error of 5 vols. % combined O₂ only causes a difference in the constant x of about 1. The reason of this is that I have not been able to obtain agreement between the O₂ determinations got by pumping out and the values obtained by Haldane's ferricyanide method. I originally became aware of this by comparing the relation between the O₂ combined by the blood and the volume of the blood corpuscles of the same blood. A. Norgaard and H. C. Gram and later H. C. Gram [1921] found about 38.5 vols. % combined O₂ in 100 cc. human blood corpuscles but V. Bie and P. Möller [1913] found about 45 vols. %, a similar quantity to that I have myself found. Later on, it appears, W. B. Cannon, J. Fraser and A. N. Hooper [1919], in oxygenated blood from patients suffering from shock, found about 43 vols. %. While Norgaard and Gram's haemoglobinometer was corrected by the ferricyanide method, Bie and Möller's apparatus was corrected by Fridericia by pumping out, just as my own results were obtained. Dr Marie Krogh was kind enough to determine the O₂ in some blood samples from man and horse with a Haldane haemoglobinometer which was very accurately calibrated by the ferricyanide method. She constantly found lower O₂ values than I did with the exhaust pump. I have no doubt whatever that too low readings are obtained under special circumstances with Haldane's ferricyanide method as shown lately by van Slyke and Stadie [1921] and I hope soon to take up this question again. F. Müller [1904] previously found the same for dog blood but considered it only applied to quite fresh blood and under unfavourable circumstances.

relating to ox and horse blood the curve which most nearly corresponded to the O_2 determinations was employed.

The value used in the calculations for $p\lambda_{(s)} + \log \Phi_a(CO_2)$ with human, ox, and horse blood at 38° was 6.190; at 37° , 6.185; at 17° , 6.334; at 18° , 6.327; at 19° , 6.320. In the case of pig and pigeon blood at 38° , 6.20 was used; with dog and rabbit blood at 19° and 18.5° , 6.35. With haemolysed blood equation (134) was employed with the following constants: at 17° , 6.325 was employed; at 18° , 6.320; at 19° , 6.315. With serum equation (121) was used and as constants at 38° , 6.165 was used; at 19° , 6.286; at 20° , 6.281; at 21° , 6.274. The results of these calculations and of the smoothing on the curves for some of the experiments carried out in the laboratory of the Finsen Institute will be found in Tables LII, LIII and LVIII and in Figs. 16, 17, 19, 20 and 21. The combination of CO_2 in *human, pigeon, horse, dog, rabbit, pig and ox blood* and in some sera can therefore be expressed by an equation of the form of (181) or (182).

It will be observed, as already mentioned, that all the curves determined in this laboratory are straight lines, allowing for experimental error. In the case of a number of the serum curves however the point corresponding to the highest CO_2 tension is too low. This seems to me only to be an indication that equilibrium had not been quite reached in these experiments as at the time I undertook them I was not aware of the deficiency of the small saturator. I have omitted these experiments in the calculus of smoothing, and in the tables this is denoted by a line drawn through them. It will be easily seen without a special note which experiments are included and which rejected. Those portions of the curves which correspond to the rejected experiments are drawn with a dotted line. In one of the tables an experiment has been excluded because it seems to be subject to an unusually large error. This experiment is put in parentheses.

The experiments with very low tensions were carried out with the large saturator electrodes using the technique described in chapter IV.

In Table LIV I have performed similar calculations for Haldane's blood (taken from Christiansen, Douglas and Haldane's [1914] paper) and in Table LVI for Davies' blood (taken from Davies, Haldane and Kennaway's [1920] paper). The curves are given in Fig. 18.

It will be noticed the CO_2 combinations corresponding to the most alkaline values are too high both in oxygenated and reduced blood—in Davies' blood the most alkaline values are too high and the most acid too low. I believe I am right in saying these deviations from a straight line are due to the fact that the technique of saturation employed is not applicable at high and low CO_2 tensions. The experiments just recorded show this and I myself had a similar failure before I became aware of the relatively great difficulties of saturation. Davies, Haldane and Kennaway's mistake in thinking the combination of CO_2 in the blood is maximal at the highest tensions is only due to equilibrium not being established. Calculations relating to J. Joffe's blood, Table LVII and Fig. 19 (from Joffe and Poulton's [1920] paper) also demonstrate

Table LII.

Pig blood (O₂-combination 21.1 vols. %). Half saturated with O₂, 38°. Calculated after K. A. Hasselbalch. $p\lambda_{(o)} + \log \Phi_a = 6.20$ in every case.

mm. Hg CO ₂	Vols. % CO ₂ combined	$pH_{(o)}$ calculated	Vols. % CO ₂ combined calculated	Difference
8.4	21.9	7.75	20.8	+1.1
(11.4)	22.7	7.64	26.5	-3.8
15.7	28.6	7.60	28.6	0.0
30.4	36.8	7.42	37.9	-1.1
30.8	36.4	7.41	38.4	-2.0
53.1	46.3	7.28	45.2	+1.1
76.1	51.8	7.17	51.0	+0.8

$$x = 51.865 \pm 0.5. \quad y = 422.76 \pm 0.5. \quad M = 1.4.$$

Pigeon blood (O₂-combination 23.7 vols. %). Half saturated with O₂, 38°. Calculated after K. A. Hasselbalch. $p\lambda_{(o)} + \log \Phi_a = 6.20$ in every case.

5.8	21.0	7.90	20.9	+0.1
18.3	35.0	7.62	35.1	-0.1
38.5	45.3	7.41	45.7	-0.4
75.0	56.2	7.21	55.9	+0.3

$$x = 50.774 \pm 0.08. \quad y = 421.96 \pm 0.08. \quad M = 0.4.$$

Dog blood (O₂-combination 19.2 vols. %) totally reduced, 19°. $p\lambda_{(o)} + \log \Phi_a = 6.35$ in every case.

2.2	21.8	8.27	22.1	-0.3
29.3	53.5	7.54	53.6	-0.1
56.4	62.4	7.32	63.1	-0.7
77.1	68.5	7.22	67.5	+1.0
127.5	76.0	7.05	74.8	+1.2
337.6	88.8	6.70	89.9	-1.1

$$x = 43.230 \pm 0.1. \quad y = 379.58 \pm 0.1. \quad M = 1.0.$$

Rabbit blood totally reduced, 18.5°. $p\lambda_{(o)} + \log \Phi_a = 6.35$ in every case.

16.0	48.6	7.76	44.4	+4.2
24.2	53.1	7.62	52.9	+0.2
41.5	63.0	7.46	62.6	+0.4
44.2	64.1	7.44	63.9	+0.2
81.7	73.8	7.23	76.6	-2.8
83.1	76.5	7.24	76.0	+0.5
368.0	107.0	6.74	106.4	+0.6

$$x = 60.838 \pm 0.4. \quad y = 516.49 \pm 0.4. \quad M = 1.5.$$

Reduced horse blood (vols. % combined O₂, 22.6), 19°.

3.0	25.2	8.24	26.1	-0.9
22.0	56.2	7.68	55.7	+0.5
39.6	66.3	7.48	66.3	0.0
48.7	69.9	7.41	70.0	-0.1
80.2	78.2	7.24	79.0	-0.8
91.5	83.5	7.20	81.1	+2.4
484.7	112.2	6.59	113.4	-1.2

$$x = 52.908 \pm 0.2. \quad y = 462.04 \pm 0.2. \quad M = 1.3.$$

Oxygenated horse blood (vols. % O₂, 25.4; 25, 1; 25, 2), 20°.

32.7	51.5	7.50	51.4	+0.1
75.2	69.4	7.24	69.4	0.0
194.5	91.1	6.92	91.4	-0.3
364.0	106.1	6.71	105.9	+0.2
558.4	116.2	6.56	116.2	0.0

$$x = 68.897 \pm 0.06. \quad y = 568.17 \pm 0.06. \quad M = 0.2.$$

Oxygenated ox blood, 38°. Hasselbalch and Warburg.

$p\lambda_{(o)} + \log \Phi_a = 6.20$ in every case.

43.5	41.2	7.31	40.7	+0.5
93.6	54.3	7.10	54.9	-0.6
150.9	63.9	6.96	64.5	-0.6
203.9	70.1	6.87	70.4	-0.3
269.4	76.5	6.79	75.8	+0.7
361.8	83.4 ¹	6.70	81.9	+2.5
692.9	101.9 ¹	6.50	95.3	+6.6

¹ O₂ deficit.

$$x = 67.458 \pm 0.03. \quad y = 533.81 \pm 0.03. \quad M = 0.7.$$

Table LIII.

K. A. Hasselbalch's half reduced blood, 38°. K. A. Hasselbalch [1916, 2]¹.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH _(s) calculated	Vols. % CO ₂ combined calculated	Difference
18.5	35.1	7.63	36.6	-1.5
22.4	36.7	7.56	40.6	-3.9
32.7	45.1	7.48	45.2	-0.1
40.9	48.2	7.41	49.3	-1.1
45.7	52.9	7.40	49.9	+3.0
50.8	57.1	7.39	50.4	+6.7
67.0	57.9	7.27	57.4	+0.5
80.7	61.3	7.21	60.9	+0.4
102.2	62.5	7.11	66.6	-4.1

$$x = 57.858 \pm 0.9. \quad y = 477.98 \pm 0.9. \quad M = 3.6.$$

E. J. Warburg's reduced blood, 38°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH _(s) calculated	Vols. % CO ₂ combined calculated	Difference
13.6	27.7	7.66	25.9	+1.8
39.5	42.8	7.36	45.9	-3.1
74.6	56.5	7.20	56.5	0.0
109.2	64.7	7.09	63.8	+0.9
544.5	99.5	6.56	99.1	+0.4

$$x = 66.567 \pm 0.5. \quad y = 535.79 \pm 0.5. \quad M = 2.1.$$

¹ The experiments were performed with blood taken on three different days and were not specially designed for the determination of the CO₂ combination curve.

Table LIV.

J. S. Haldane's oxygenated blood, 37°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH _(s) calculated	Vols. % CO ₂ combined calculated	Difference
2.6	14.9	8.15	3.8	+11.1
8.7	25.7	7.84	23.0	+2.7
18.9	35.8	7.64	35.5	-0.3
37.7	48.6	7.46	46.6	+2.0
44.1	49.4	7.40	50.4	-1.0
41.7	48.2	7.42	49.1	-0.9
56.1	54.5	7.33	54.7	-0.2
73.3	58.1	7.25	59.7	-1.6
78.5	60.3	7.23	60.9	-0.6
105.2	68.1	7.15	65.8	+2.3

$$x = 62.016 \pm 0.5. \quad y = 509.32 \pm 0.5. \quad M = 1.6.$$

J. S. Haldane's reduced blood, 37°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH _(s) calculated	Vols. % CO ₂ combined calculated	Difference
2.15	15.3	8.24?	4.2	+11.1
7.75	30.3	7.94	23.6	+6.7
8.1	30.0	7.92	24.9	+5.1
17.9	40.4	7.69	39.8	+0.6
37.9	53.5	7.48	53.4	+0.1
58.2	60.5	7.34	62.5	-2.0
74.2	65.3	7.27	67.0	-1.7
78.7	69.9	7.27	67.0	+2.9
111.1	75.4	7.15	74.8	+0.6

$$x = 64.731 \pm 0.7. \quad y = 537.60 \pm 0.7. \quad M = 1.8.$$

Table LV.

T. R. Parsons' oxygenated blood, 37°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH ^(s) calculated	Vols. % CO ₂ combined calculated	Difference
5.7	21.3	7.93	21.4	-0.1
10.1	30.0	7.83	26.2	+3.8
19.6	34.4	7.59	37.6	-3.2
33.4	41.5	7.43	45.2	-3.7
37.4	43.4	7.40	46.6	-3.2
72.1	59.9	7.25	53.7	+6.2

$$x = 47.449 \pm 2.4. \quad y = 397.70 \pm 2.4. \quad M = 5.4.$$

T. R. Parsons' reduced blood, 37°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH ^(s) calculated	Vols. % CO ₂ combined calculated	Difference
5.7	23.8	7.96	23.0	+0.8
8.1	27.4	7.87	27.7	-0.3
10.1	31.5	7.83	29.6	+1.9
19.6	39.9	7.64	39.8	+0.1
33.4	47.8	7.48	48.1	-0.3
37.3	47.3	7.42	51.3	-4.0
55.3	55.1	7.31	57.0	-1.9
72.1	64.4	7.26	59.6	+4.8

$$x = 52.319 \pm 0.7. \quad y = 439.48 \pm 0.7. \quad M = 3.0.$$

Table LVI.

H. W. Davies' blood, 38°.

mm. Hg CO ₂	Vols. % CO ₂ combined	pH ^(s) calculated	Vols. % CO ₂ combined calculated	Difference
8.3	26.3	7.88	6.0	+20.3
9.02	29.6	7.90	4.3	+25.3
11.2	28.7	7.78	14.5	+14.2
19.3	35.5	7.63	27.2	+ 8.3
29.8	41.4	7.50	38.2	+ 3.2
38.9	46.4	7.44	43.3	+ 3.1
39.0	45.5	7.42	44.9	+ 0.6
39.1	43.1	7.39	47.5	- 4.4
46.6	47.3	7.35	50.9	- 3.6
51.3	53.2	7.36	50.0	+ 3.2
64.9	57.4	7.29	55.9	+ 1.5
68.5	60.0	7.28	56.8	+ 3.2
76.7	62.0	7.25	59.3	+ 2.7
89.9	65.7	7.20	63.5	+ 2.2
102.9	66.5	7.15	67.8	- 1.3
202.0	81.8	6.93	86.3	- 4.5
215.0	82.6	6.91	88.1	- 5.5
411.0	97.3	6.69	106.7	- 9.4
455.0	97.6	6.65	110.1	-12.5
497.0	95.7	6.60	114.4	-18.7

$$x = 84.657 \pm 0.4. \quad y = 673.09 \pm 0.4. \quad M = 4.8.$$

Table LVII.

J. Joffe's defibrinated oxygenated blood, 4. viii.-28. xi. 1919.
Joffe and Poulton, Tables V and VII.

mm. Hg CO ₂	Vols. % CO ₂ combined	PH ^(a) calculated	Vols. % CO ₂ combined calculated	Difference
4.0	13.7	7.93	5.4	+ 8.3
5.0	16.8	7.92	6.1	+10.7
12.9	23.8	7.65	24.0	- 0.2
19.7	29.4	7.54	31.3	- 1.9
24.9	36.0	7.53	31.9	+ 4.1
34.6	40.0	7.42	39.3	+ 0.7
44.4	44.8	7.36	43.2	+ 1.6
50.2	44.1	7.30	47.2	- 3.1
58.0	47.6	7.26	49.9	- 2.3
65.3	52.6	7.25	50.5	+ 2.1
67.8	53.9	7.25	50.5	+ 3.4
82.2	56.0	7.17	55.8	+ 0.2
110.3	63.3	7.10	60.5	+ 2.8
149.0	62.6	6.96	69.8	- 7.2
156.0	67.1	6.97	69.1	- 2.0
157.0	70.3	6.98	68.4	+ 1.9
180.0	68.5	6.91	73.1	- 4.6
376.3	90.0	6.69	87.7	+ 2.3
610.0	100.3	6.53	98.3	+ 2.0

$$x = 66.345 \pm 0.16. \quad y = 531.52 \pm 0.16. \quad M = 3.2.$$

J. Joffe's defibrinated reduced blood, 4. viii.-9. x. 1920.
Joffe and Poulton, Table VII.

9.9	27.9	7.81	20.1	+7.8
15.0	34.4	7.72	26.3	+8.1
19.4	35.3	7.61	34.0	+1.3
27.6	40.1	7.50	41.6	- 1.5
30.9	44.1	7.50	41.6	+2.5
36.8	47.9	7.45	45.1	+2.8
41.7	46.0	7.37	50.6	-4.6
47.0	50.4	7.36	51.3	-0.9
50.5	49.8	7.33	53.4	-3.6
55.7	52.9	7.31	54.8	-1.9
70.1	55.6	7.23	60.4	-4.8
76.8	62.1	7.23	60.4	+1.7
77.7	64.0	7.24	59.7	+4.3
92.7	67.6	7.19	63.1	+4.5

$$x = 69.475 \pm 0.7. \quad y = 562.65 \pm 0.7. \quad M = 3.6.$$

that the most alkaline values are too high but the acid values are correct. Parsons' [1917] blood gives values scattered irregularly about a straight line (Table LV and Fig. 19).

The constant x , which is the tangent to the angle the straight line makes with the axis of the abscissa measured in the first or third quadrant, is for

Davies' oxygenated blood	...	84.7
Haldane's " " "	...	62.0
" reduced " "	...	64.7
Hasselbalch's half oxygenated blood	...	57.9
Joffe's oxygenated blood	...	66.3
" reduced "	...	69.5
Parsons' oxygenated "	...	47.4
" reduced "	...	52.3
Warburg's " "	...	66.6

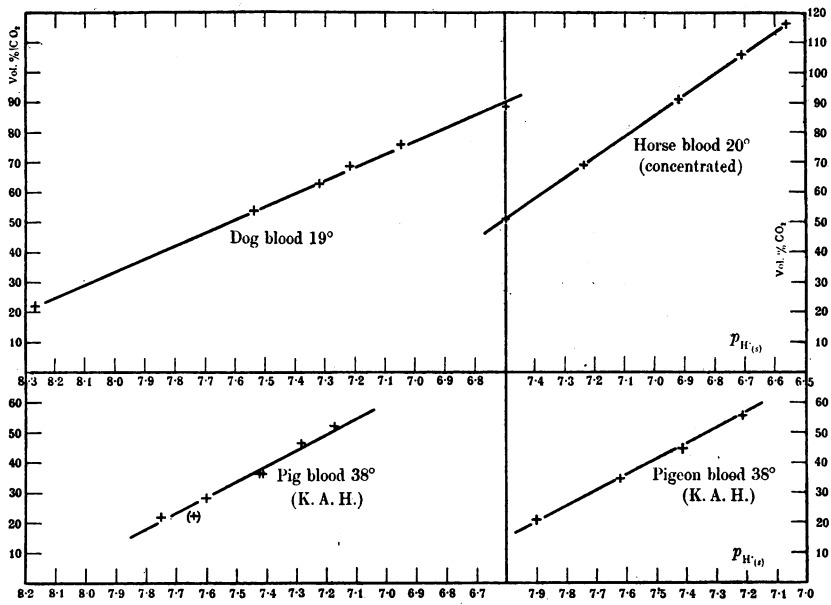


Fig. 16.

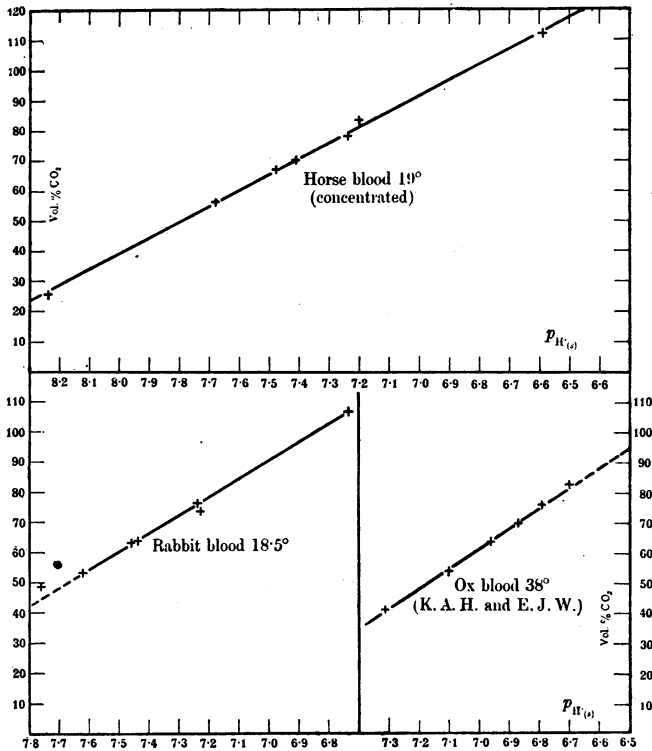


Fig. 17.

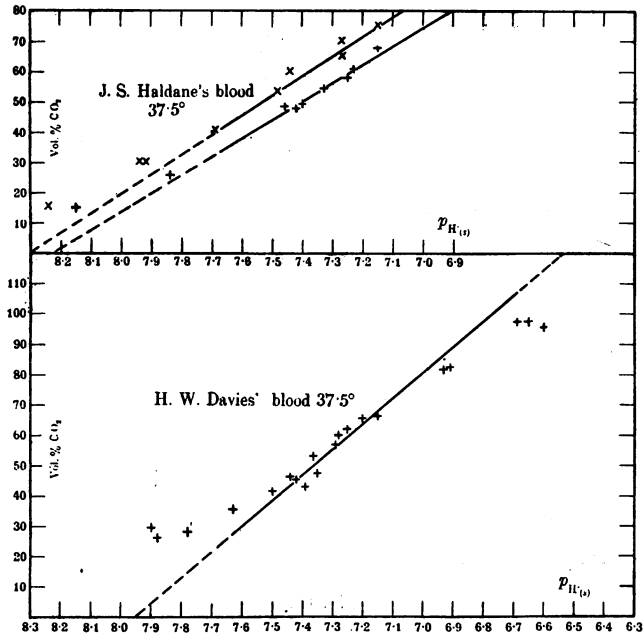


Fig. 18.

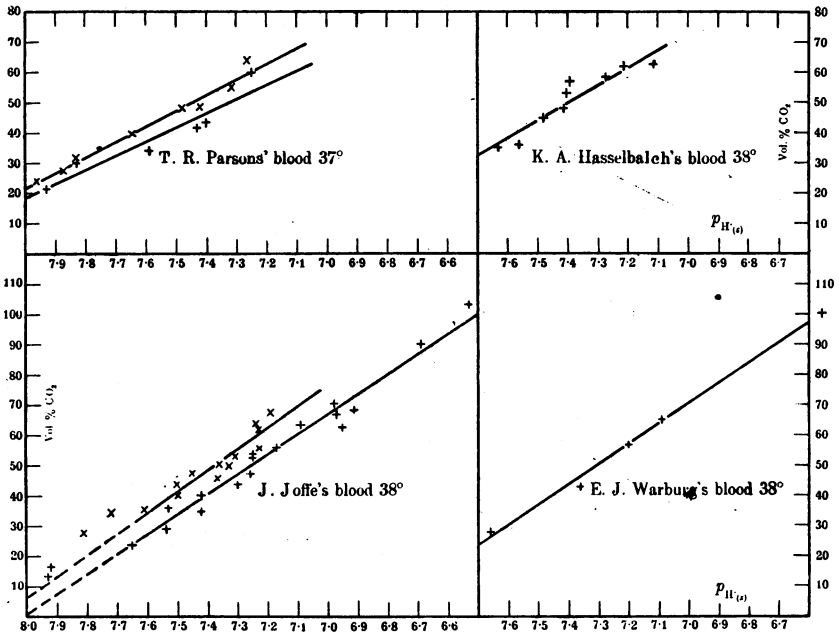


Fig. 19.

It will be observed in the first place that the constant varies from blood to blood. The greater part of the difference is undoubtedly due to variations in the amount of haemoglobin as Hasselbalch [1916, 2] has pointed out and as is evident from the experiments of Schmidt [1867], Zuntz [1867, 1868], Setschenow [1879], Jaquet [1892], Bohr [1905] and many others, but the examination of numbers of curves has shown that this cannot be the sole determining factor, as Peters, Jr. and Barr [1921] have also indicated from a rather different point of view (Peters and Barr's curves are constructed as P_{CO_2} -vols. % total CO_2 curves). It will also be seen that x is a little greater in reduced blood than in the corresponding oxygenated blood. The difference is certainly a real one and is of almost the same magnitude in Haldane's, Joffe's and Parsons' blood. L. J. Henderson [1920, 1] has recently demonstrated the same thing graphically and he has discussed the cause in a paper dealing with the influence of oxygen on the combined CO_2 of the blood. This paper seems to be of very considerable interest although I do not agree with the author in all his views but as I have not yet succeeded in quite clearing up several of the problems I shall only refer those interested to the original article.

By plotting a p_{H} -vols. % combined CO_2 curve it is possible to form a good idea of the reliability of the curve. I have done this for a number of Haggard and Henderson's experiments and for some of Peters, Barr and Rule's also. It appears especially in the case of Haggard and Henderson's, that the technique is not so good as the authors believe and it seems to me problematical whether the accuracy is sufficient to draw any conclusion about the tension with which the blood is in equilibrium from the total amount of CO_2 in the blood, as these authors do. Haggard and Henderson's [1920, 1] discovery that there is no difference between the combined CO_2 in oxygenated and reduced oxalate blood, and that the combined CO_2 of the blood decreases irreversibly by blowing air through it vigorously, is presumably due to experimental error.

I must now discuss some experiments published in the last few years by H. Straub and Klothilde Meier [1918-1920]. They have unfortunately gained some recognition, *e.g.* by L. Michaelis¹ and by Parsons. But their results are so surprising, that Joffe and Poulton [1920], Peters, Barr and Rule [1921] for example have announced that they are sceptical. Straub and Meier's chief claim is that they have shown the combined CO_2 in the blood does not increase before a reaction of about $p_{\text{H}} \cdot 6.70$ is reached when suddenly (in an interval of $p_{\text{H}} \cdot 0.01$) an amount of CO_2 as great as the oxygen capacity is combined by the blood and then all further combination ceases. In haemo-

¹ Michaelis and Airila have recently [1921] shown by cataphoresis experiments that the change of haemoglobin varies uniformly with the reaction over a very large range of reaction and is thus greatly at variance with Straub and Meier's assertions about the ionisation of haemoglobin. The conclusion may be drawn from these interesting experiments that the combination of CO_2 in haemoglobin solutions can be represented by a curve which, if not a straight line, is a very close approximation thereto, in a p_{H} -vols. % diagram over a very considerable range of reaction.

lysed blood the sudden change is at p_H 7.00 and is of the same extent. They believe serum is quite unable to combine with CO_2 in quantities varying with the pressure. Straub and Meier claim to have shown that various substances shift this sudden change backwards and forwards, and they have formed a theory which for extravagance is only surpassed by the obvious imperfection of their technique. With regard to these authors I cannot suppress my surprise that they really seem to be quite ignorant of the older, and as far as the present question is concerned, of the more recent literature on the combined CO_2 of the blood and it is to me completely incomprehensible that they have not paid more attention to their technique.

As an illustration of Straub and Meier's technique I will give one of their experiments [1920, 2, p. 250].

Experiment 9. 38°.

mm. Hg CO_2	Vols. % CO_2 total
16.8	15.1
50.2	19.6
72.6	30.6
153.3	38.7
41.4	35.9
64.0	34.9

The only possible explanation is, as the authors themselves have realised, that equilibrium has not been reached again at the low CO_2 tension. Instead of examining their technique they employ the experiment in propounding one of their extraordinary theories, and they fail to see that p_H cannot be calculated by Henderson and Hasselbalch's equation if equilibrium in the system has not been attained. The following similar experiment shows that equilibrium can easily be attained.

Ox blood slightly concentrated by centrifuging. Combined O_2 , 22.8 vols. %. 70 cc. blood in the large saturator.

21°.	mm. Hg CO_2	Vols. % CO_2 total
First	29.0	47.9 after 30 mins.
Then	740	185.5 " 30 "
"	"	184.3 " 65 "
Afterwards	29.0	49.8 " 30 "
"	"	47.8 " 60 "
"	"	47.7 " 120 "

In Table LVIII and in Figs. 20 and 21 experiments and algebraic smoothing will be found referring to different sera. These curves also appear to be straight lines although a number of points at high CO_2 tensions fall a little below the curve. It should however be noted that deviations from a straight line course are more difficult to detect than in the case of the blood curves on account of the relatively small value of x .

That serum and plasma really form a dissociable combination with CO_2 is raised beyond all doubt and has already been demonstrated by Setschenow [1879] and by Jaquet [1892], and later by Hasselbalch [1916, 2] and Hasselbalch and Warburg [1918] among others. It is also quite certain that varying

carbonic acid is combined as bicarbonate, as is shown by Loewy and Zuntz [1894, 1] and by Gürber's [1895, 2] old diffusion experiments. Gürber even claimed to have proved that CO_2 quantitatively was present as bicarbonate, a conclusion which is strongly supported by the determinations of $p\lambda_{(s)}$ in chapter VI of this work.

Table LVIII.

Ox serum 38°. Hasselbalch and Warburg. I.				
mm. Hg CO_2	Vols. % CO_2 combined	$p\text{H}'_{(s)}$ calculated	Vols. % CO_2 combined calculated	Difference
41.3	54.4	7.42	54.1	+0.3
74.5	58.5	7.20	58.6	-0.1
140.5	62.2	6.95	63.7	-1.5
203.0	67.5	6.82	66.4	+1.1
271.5	68.9	6.71	68.6	+0.3
710.0	76.3	6.33	76.4	-0.1
$x = 20.507 \pm 0.2$. $y = 206.23 \pm 0.2$. $M = 1.0$.				
Ox serum 19° (serum B). Hasselbalch and Warburg. II.				
19.5	61.6	7.73	Unsuitable for calculation (see Fig. 20)	
79.8	69.6	7.17		
182.0	73.0	6.82		
378.3	73.7	6.52		
Horse serum 38°. III.				
23.5	63.8	7.73	63.9	-0.1
24.6	63.4	7.71	64.1	-0.7
45.6	67.5	7.47	67.0	+0.5
104.6	71.8	7.14	71.0	+0.8
430.3	77.3	6.57	77.8	-0.5
$x = 12.016 \pm 0.2$. $y = 156.76 \pm 0.2$. $M = 0.7$.				
Horse serum 38°. IV.				
10.4	58.1	8.05	57.9	+0.2
35.2	63.3	7.56	63.6	-0.3
106.7	69.2	7.11	68.9	+0.3
130.7	69.7	7.03	69.8	-0.1
535.6	74.9	6.42	76.9	-2.0
$x = 11.644 \pm 0.1$. $y = 151.67 \pm 0.1$. $M = 0.3$.				
Ox serum 38°. V.				
22.7	62.6	7.74	64.2	-1.6
59.5	69.0	7.37	69.4	-0.4
103.7	74.7	7.16	72.4	+2.3
510.7	80.1	6.50	81.6	-1.5
$x = 14.012 \pm 0.6$. $y = 172.69 \pm 0.6$. $M = 2.3$.				
Human serum 38°. VI.				
15.2	57.2	7.88	58.7	-1.5
18.3	60.7	7.82	59.5	+1.2
84.5	68.7	7.21	68.2	+0.5
562.1	78.7	6.45	79.0	-0.3
$x = 14.237 \pm 0.3$. $y = 170.83 \pm 0.3$. $M = 1.4$.				
Horse serum 19°. VII.				
12.7	55.8	7.86	56.5	-0.7
18.7	58.2	7.71	57.9	+0.3
41.2	62.6	7.40	60.9	+1.7
119.6	64.4	6.95	65.2	-0.8
512.5	71.1	6.36	70.9	+0.2
$x = 9.5901 \pm 0.2$. $y = 131.87 \pm 0.2$. $M = 1.2$.				

Table LVIII (continued)

Horse serum (19.5°) 20°. VIII.

mm. Hg. CO ₂	Vols. % CO ₂ combined	pH _(t) calculated	Vols. % CO ₂ combined calculated	Difference
13.1	55.6	7.86	56.7	-1.1
34.1	61.1	7.48	60.5	+0.6
83.1	65.3	7.13	64.0	+1.3
573.7	71.4	6.32	72.2	-0.8

$$x = 10.025 \pm 0.3. \quad y = 135.51 \pm 0.3. \quad M = 1.4.$$

Ox serum 19°. IX.

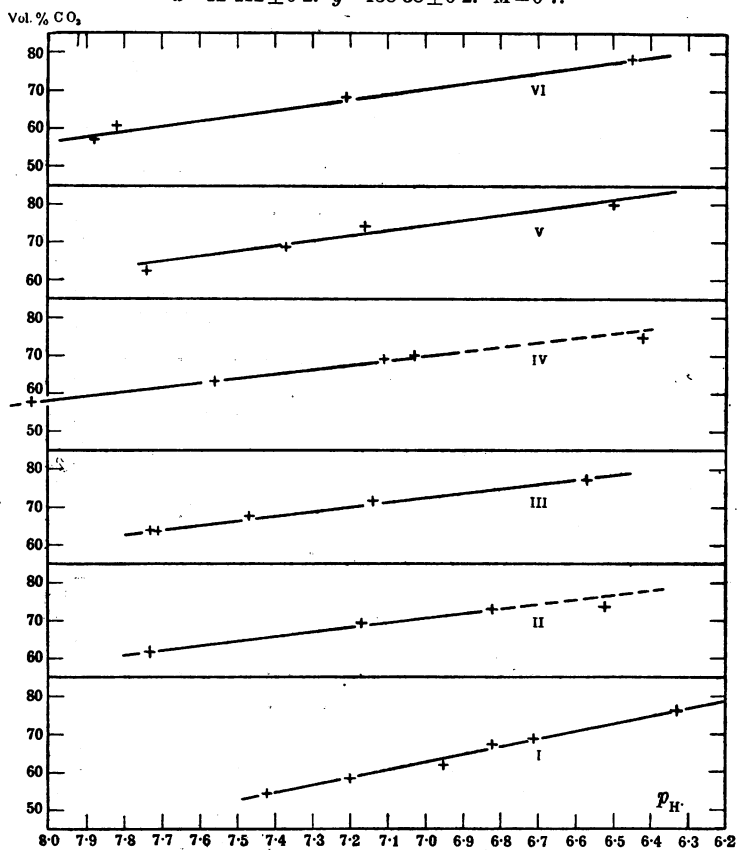
16.0	58.5	7.78	59.3	-0.8
43.1	64.7	7.40	63.9	+0.8
118.6	69.4	6.99	68.9	+0.5
545.9	75.9	6.37	76.4	-0.5

$$x = 12.127 \pm 0.2. \quad y = 153.65 \pm 0.2. \quad M = 0.9.$$

Ox serum 21°. X.

16.1	61.8	7.81	61.4	+0.4
40.5	66.0	7.44	66.0	0.0
40.6	65.3	7.44	66.0	-0.7
70.2	68.5	7.22	68.8	-0.3
122.9	71.9	7.00	71.5	+0.4
595.0	75.4	6.33	79.8	-4.4

$$x = 12.412 \pm 0.2. \quad y = 158.38 \pm 0.2. \quad M = 0.7.$$

Fig. 20. The combination of CO₂ by sera.

In a paper on the neutrality of the blood W. M. Bayliss [1919] has recently advanced the opinion that the proteins of serum and plasma could not function as ampholytes at the reactions obtaining in my experiments and in the above mentioned papers. I do not agree with Bayliss on this point and as the subject is of prime importance for the problems we are discussing I shall attempt briefly to dispute his statements.

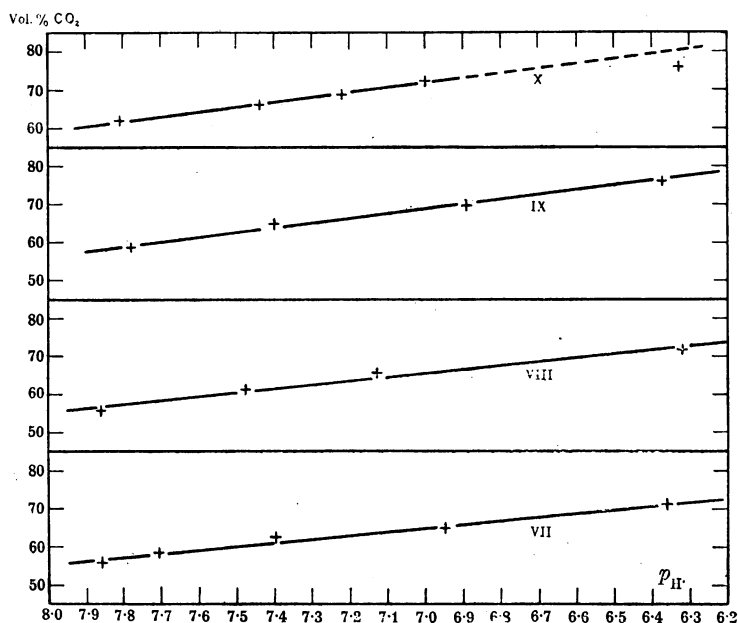


Fig. 21. The combination of CO₂ by sera.

Bayliss¹ asserts that serum does not combine with CO₂ in a reversible manner at the usual CO₂ tensions, supporting this by an experiment without doubt wrongly interpreted. It is obvious from the experiment that the variable combination—which Bayliss considers unimportant—is of the same order as that found by other authors. Bayliss further maintains that proteins do not act as buffers at reactions in the neighbourhood of the neutral point. This result is absolutely opposed to the finding of L. J. Henderson [1909–1910] and T. B. Robertson [1908–1910, 1912] with serum globulin and to the above mentioned experiments. The reason he has come to this erroneous conclusion must be that he has overrated the accuracy with which the reaction of plasma can be determined colorimetrically. In the same paper W. M. Bayliss has concluded by analogy that *haemoglobin* cannot act as a buffer. This analogy is however unjustifiable, quite apart from whether the experiments with plasma are correct or not, because the ampholytic character of the various

¹ Mukai (*J. Physiol.* 1921, 55, 356) has quite recently, in Bayliss' laboratory, found that serum combines with variable amounts of CO₂ of a similar order to those found by earlier authors.

proteins is very different as is undoubtedly shown by the work of Robertson [1912] and his collaborators, and by the studies of Pauli [1920] and his co-workers.

In Table LIX and Fig. 22 the calculations from Campbell and Poulton's [1920] experiments with dialysed haemoglobin solution at 38° will be found. The corresponding curves are here also straight lines allowing for experimental error. As 2226C_I was 91.5 vols. % in the experiments the isoelectric point is 7.14. This is, as Campbell and Poulton [1920] themselves have remarked, considerably more alkaline than Michaelis and Takahashi [1910] found at room temperature the isoelectric point according to these observers being *p*_H (Bjerrum) 6.79. Now it can be shown from a glance at the CO₂ combination curves—as Hasselbalch [1916, 2] has pointed out—that the isoelectric point for haemoglobin must be more acid at body temperature than at room temperature (the difference is about 0.30), so that the difference between Campbell and Poulton's and Michaelis' results is real¹.

Table LIX. Dialysed solution of haemoglobin (Haldane 52) with 0.041*n* NaHCO₃, 38°; *p*λ_(*m*) = 6.23. Calculated after J. M. H. Campbell and E. P. Poulton.

mm. Hg CO ₂	Vols. % CO ₂ combined	<i>p</i> _H calculated	Vols. % CO ₂ combined calculated	Difference
4.8	52.3	8.40	36.2	+ 16.1
4.9	52.1	8.39	36.6	+ 15.5
11.0	57.5	8.08	50.3	+ 7.2
21.9	63.3	7.83	61.2	+ 2.1
40.7	72.9	7.62	70.4	+ 2.5
43.6	76.5	7.61	70.9	+ 5.6
50.7	75.5	7.54	70.0	+ 5.5
52.5	70.2	7.47	77.0	- 6.8
78.1	74.1	7.34	82.8	- 8.7
85.5	79.8	7.34	82.8	- 3.0
90.0	77.1	7.30	84.5	- 7.4
110.5	92.3	7.29	85.0	+ 7.3
119.8	86.3	7.22	88.0	- 1.7
151.4	93.5	7.16	90.7	+ 2.8
221.9	99.1	7.02	96.8	+ 2.3
226.7	97.8	7.00	97.7	+ 0.1
310.4	101.9	6.88	103.0	- 1.1
338.0	107.0	6.87	103.4	+ 3.6
435.3	110.0	6.77	107.8	+ 2.2
596.4	112.2	6.64	113.5	- 1.3

$$x = 43.959 \pm 0.2. \quad y = 405.41 \pm 0.2. \quad M = 4.5.$$

Some experiments I have performed give an indication of the position of the isoelectric point. According to Hardy the agglutination optimum of

¹ T. R. Parsons and Winifred Parsons (*Biochem. Zeitschr.* 1921, 126, 108) have recently pointed out that Campbell and Poulton's haemoglobin solutions must have contained inorganic salts because when they incinerated the haemoglobin they found more ash than could have come from the iron alone. Calculating the amount of HCO₃ which would be balanced by metallic ions on the assumption the excess of ash was K₂CO₃ we get 30 vols. % and the isoelectric point is therefore about 6.5, which agrees with Michaelis' determination when we take the difference of temperature into account.

a colloid is at the isoelectric point. The crystallising out of haemoglobin at high CO_2 tensions (after saponin haemolysis) mentioned in chapter IV never began at reactions more alkaline than $p_{\text{H}} 7.10$ at room temperature under the conditions of the experiment, but the ease with which crystallisation was set going increased until $p_{\text{H}} 6.80$ was reached which supports Michaelis' result. Should further experiments confirm the difference between the isoelectric point estimated from the combined CO_2 and from the other method, it will indicate that some CO_2 is adsorbed or combined in a complex manner with the haemoglobin.

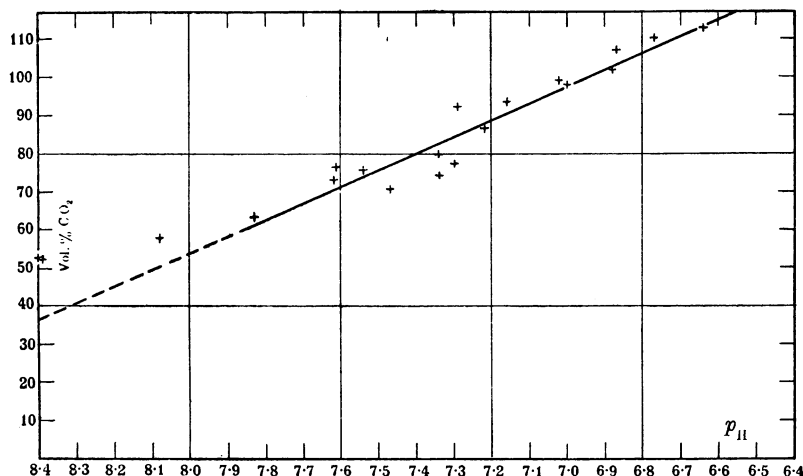


Fig. 22. CO_2 combined by dialysed haemoglobin (with the addition of $0.041n \text{ NaHCO}_3$). (J. M. H. C. & E. P. P.)

With the help of the relations developed in this chapter, which found their simplest expression in (173) and (176), it will be possible to investigate whether all the reversibly combined CO_2 is present as bicarbonate. Before reporting the experiments I have made in this connection I would draw attention to the fact that an error in $p\lambda_{(s)}$ or $p\lambda_{(m)}$ or in the correction taken from Fig. 10 is without significance here, because it will influence the estimation of the hydrogen ion exponent in the original and in the curve displaced by the addition of alkali to the same extent and thus the relative positions of the curves will not be altered.

The experiments were carried out as follows: blood or saponin haemolysate ($\frac{1}{2}$ % saponin) which was slightly concentrated by pipetting off serum was sloped in a 100 cc. measuring flask so that it filled about half of it. With a pipette either 5 cc. $n/2 \text{ NaCl}$ solution or 5 cc. $n/2 \text{ NaHCO}_3$ solution was then added during shaking, the titre of which was determined beforehand by the exhaust pump. Two drops of octyl alcohol were next added to prevent frothing after which the flask was filled up with blood (or haemolysate).

With the mixtures thus prepared CO_2 combination curves were constructed with the help of the large saturator. The calculations were made as before, and the results are given in Table LX and in Figs. 23, 24 and 25.

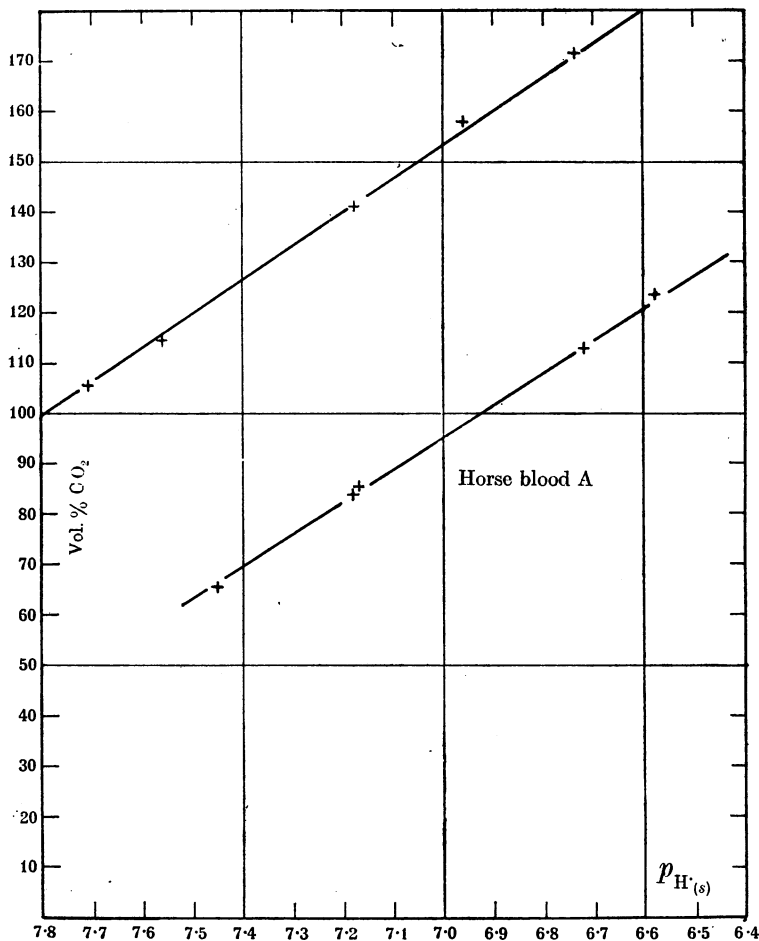


Fig. 23.

Let us first compare the constant α .

	Lower curve	Upper curve	Difference
Horse blood A	66.9 ± 0.3	69.2 ± 0.3	2.3 ± 0.4
Horse blood haemolysate B	87.0 ± 0.6	89.2 ± 0.5	2.2 ± 0.7
Ox " " C	57.9 ± 1.1	57.8 ± 0.9	-0.1 ± 1.4
Horse " " D	81.3 ± 0.1	83.3 ± 0.3	2.0 ± 0.3
" " " E	64.0 ± 0.1	70.3 ± 0.3	6.3 ± 0.3

Table LX.

Temp.	mm. Hg		Vols. % combined		$pH^{(c)}$ calculated	Vols. % CO ₂ combined calculated	Difference vols. %
	CO ₂	O ₂	CO ₂	O ₂			
Defibrinated horse blood A. Curve I. $\Psi = 11$.							
17	43.5	147.0	65.6	26.5	7.45	65.6	0.0
"	99.8	135.5	83.8	26.4	7.18	83.7	+ 0.1
"	104.6	—	85.4	26.4	7.17	84.3	+ 1.1
"	358.8	—	112.7	—	6.72	114.4	- 1.7
"	537.8	—	123.5	—	6.59	123.1	+ 0.4
Mean 26.4							
$x = 66.883 \pm 0.3$. $y = 563.87 \pm 0.3$. $M = 1.2$. Theoretical distance 57.0.							
Curve II.							
17	40.4	147.6	105.6	26.9	7.71	104.9	+ 0.7
"	59.9	143.7	114.5	26.5	7.56	115.3	- 0.8
"	168.0	120.5	140.9	26.4	7.18	141.6	- 0.7
"	303.8	—	158.1	—	6.96	156.8	+ 1.3
"	522.4	—	171.5	—	6.74	172.0	- 0.5
Mean 26.6							
$x = 69.172 \pm 0.3$. $y = 638.24 \pm 0.3$. $M = 1.1$.							
Saponin haemolysed horse blood B. Curve I. $\Psi = 11$.							
18	14.4	—	33.6	26.2	7.60	31.3	+ 2.3
"	45.2	—	54.2	25.9	7.31	56.5	- 2.3
"	84.8	—	69.3	—	7.15	70.4	- 1.1
"	359.3	—	107.4	—	6.71	108.7	- 1.3
"	536.8	—	121.4	—	6.59	119.2	+ 2.2
"	137.4	—	75.0	—	6.97	86.1	- 11.1 ¹
"	359.3	—	92.6	—	6.64	114.8	- 22.2 ¹
¹ Crystallisation.							
$x = 87.033 \pm 0.6$. $y = 692.70 \pm 0.6$. $M = 2.5$. Theoretical distance 53.0.							
Curve II.							
18	20.3	—	69.4	—	7.77	68.3	+ 1.1
"	45.6	—	89.0	—	7.52	90.6	- 1.6
"	70.6	—	102.5	—	7.40	101.3	+ 1.2
"	141.6	—	120.8	—	7.16	122.7	- 1.9
"	360.3	—	150.7	—	6.86	149.5	+ 1.2
$x = 89.176 \pm 0.5$. $y = 761.21 \pm 0.5$. $M = 1.8$.							
Saponin haemolysed ox blood C. Curve I. $\Psi = 8$.							
19	19.4	—	38.7	20.6	7.54	37.7	+ 1.0
"	60.2	—	56.7	—	7.21	56.8	- 0.1
"	394.1	—	93.6	—	6.62	91.0	+ 2.6
"	166.9	—	73.6	—	6.86	77.1	- 3.5
$x = 57.899 \pm 1.1$. $y = 474.27 \pm 1.1$. $M = 3.2$. Theoretical distance 54.5.							
Curve II.							
19	44.4	—	91.1	20.3	7.55	91.6	- 0.5
"	398.5	—	135.4	—	6.77	136.6	- 1.2
"	164.9	—	120.1	—	7.10	117.6	+ 2.5
"	82.3	—	102.9	—	7.34	103.7	- 0.8
$x = 57.772 \pm 0.9$. $y = 527.75 \pm 0.9$. $M = 2.1$.							
Saponin haemolysed horse blood D. Curve I. $\Psi = 11$.							
18	18.3	133.9	36.7	25.0	7.53	36.4	+ 0.3
"	18.3	133.9	36.5	24.4	7.53	36.4	+ 0.1
17	52.9	—	57.8	—	7.26	58.3	- 0.5
"	91.9	—	70.3	—	7.11	70.5	- 0.2
18	531.8	—	114.6	—	6.57	114.4	+ 0.2
17	153.4	—	81.5	—	6.95	83.5	- 2.0 ¹
"	348.0	—	97.4	—	6.67	106.3	- 8.9 ¹
¹ Crystallisation.							
$x = 81.260 \pm 0.1$. $y = 648.25 \pm 0.1$. $M = 0.5$. Theoretical distance 54.5.							

Table LX (*continued*)

Temp.	mm. Hg		Vols. % combined		PH ^(a) calculated	Vols. % CO ₂ combined calculated	Difference Vols. %
	CO ₂	O ₂	CO ₂	O ₂			
Curve II.							
18	532.4	—	157.0	—	6.70	157.3	- 0.3
17	16.8	—	66.9	—	7.82	64.0	+ 2.9
"	52.6	—	91.9	—	7.47	93.2	- 1.3
"	72.8	—	100.0	—	7.36	102.3	- 2.3
"	147.5	—	120.4	—	7.14	120.7	- 0.3
"	515.9	—	158.3	—	6.71	156.5	+ 1.8
"	320.0	—	141.8	—	6.87	143.2	- 1.4 ¹

¹ Crystallisation.

$$x = 83.322 \pm 0.3. \quad y = 715.57 \pm 0.3. \quad M = 1.6.$$

Saponin haemolysed horse blood E. Curve I. $\Psi = 11$.

18	19.8	—	41.7	25.7	7.55	41.7	0.0
"	61.7	—	61.6	—	7.23	62.2	- 0.6
17	80.0	—	68.0	—	7.15	67.3	+ 0.7
"	155.6	—	80.9	—	6.94	80.8	+ 0.1
"	342.3	—	97.1	—	6.68	97.4	- 0.3
"	545.9	—	107.8	—	6.52	107.7	+ 0.1

$$x = 64.052 \pm 0.1. \quad y = 525.29 \pm 0.1. \quad M = 0.5. \quad \text{Theoretical distance } 54.5.$$

Curve II.

18	22.1	—	78.6	26.0	7.78	77.1	+ 1.5
"	101.9	—	111.3	—	7.27	113.0	- 1.7
"	185.9	—	126.1	—	7.06	127.8	- 1.7
17	301.3	—	140.0	—	6.89	139.8	+ 0.2
"	514.8	—	153.3	—	6.70	153.1	+ 0.2
"	512.3	—	154.7	26.1	6.70	153.1	+ 1.6

$$x = 70.335 \pm 0.3. \quad y = 624.37 \pm 0.3. \quad M = 1.6.$$

Apart from ox blood haemolysate C, which appears to be subject to a far greater fortuitous error than the other members of the series, the upper curves all exhibit a rather steeper ascent than the lower ones. Although we should be very cautious, as previously explained, in drawing definite conclusions from the size of the mean error about an actual discrepancy between the constants it is extremely probable the upper curve really has a somewhat steeper course than the lower. In accordance with this the equation for the combination of CO₂ in the blood and haemolysate should not be

$$(173) \quad B = (C_I - C_V f(a_{H'})) 2226,$$

but

$$(176) \quad B = (C_I - C_V f(a_{H'}) - f_1(P_{CO_2})) 2226,$$

and therefore some CO₂ should be combined in blood in some other form than bicarbonate. There is however an alternative explanation which seems to me more probable. As will be remembered it was explained on p. 280 that the difference between the curves at no place could be less than 2226*b*, which is the value entered in the tables as the "theoretical distance." We will now investigate how far this is correct.

			B		Difference		Theoretical distance
			At p_{H^+}	At p_{H^+}	At p_{H^+}	At p_{H^+}	
			7.50	6.70	7.50	6.70	
Horse blood A.	Upper curve		119.5	174.8			
	Lower "		63.0	114.3	56.5	60.5	57.0
"	haemolysate B.	Upper "	92.4	163.8	52.4	55.2	53.0
	Lower "		40.0	108.6			
Ox	"	C. Upper	94.3	140.7	54.3	54.3	54.5
	Lower "		40.0	86.4			
Horse	"	D. Upper	90.7	157.3	51.9	53.5	54.5
	Lower "		38.8	103.8			
"	"	E. Upper	96.9	153.1	52.0	57.0	54.5
	Lower "		44.9	96.1			

There seems therefore to be an inclination to obtain too small differences in the most alkaline reactions. In the case of the haemolysates D and E this seems to exceed the experimental error as the latter may be estimated as below 2 vols. % of the difference between the curves, determined by five points or more. (The standard deviation for all the determinations with the

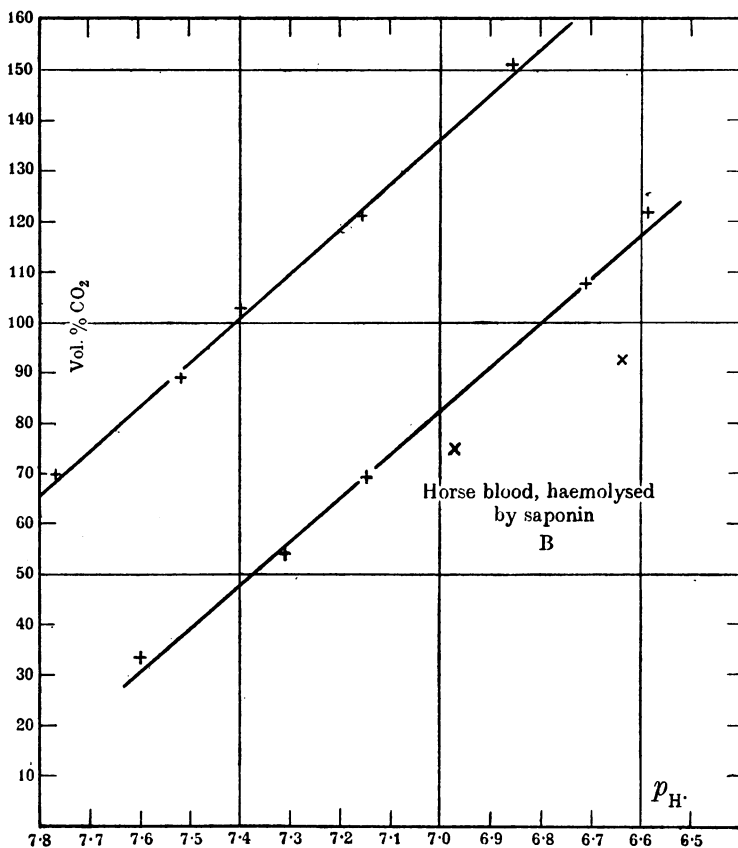


Fig. 24.

exception of the horse blood haemolysate D is 1.2 vols. %.) A possible explanation of the phenomenon is that on the addition of bicarbonate the blood was very little changed so that before the bicarbonate was completely mixed with the blood a slight decomposition of the proteins took place with the formation of new acid radicles.

The result of these experiments is therefore that it is probable that CO_2 is combined in the blood only as carbonic acid, that is to say as bicarbonate ion, but that the possibility cannot be entirely excluded that small quantities may be combined in other ways.

In the haemolysates B and D haemoglobin crystallised out in the acid reactions. It will be noticed that the combination of CO_2 decreases when crystallisation takes place but it must be remembered we cannot be sure of getting a homogeneous sample of the whole system when the crystal phase is in process of formation. Setschenow [1879] has observed a similar decrease of the combined CO_2 of horse blood when the haemoglobin crystallised out.

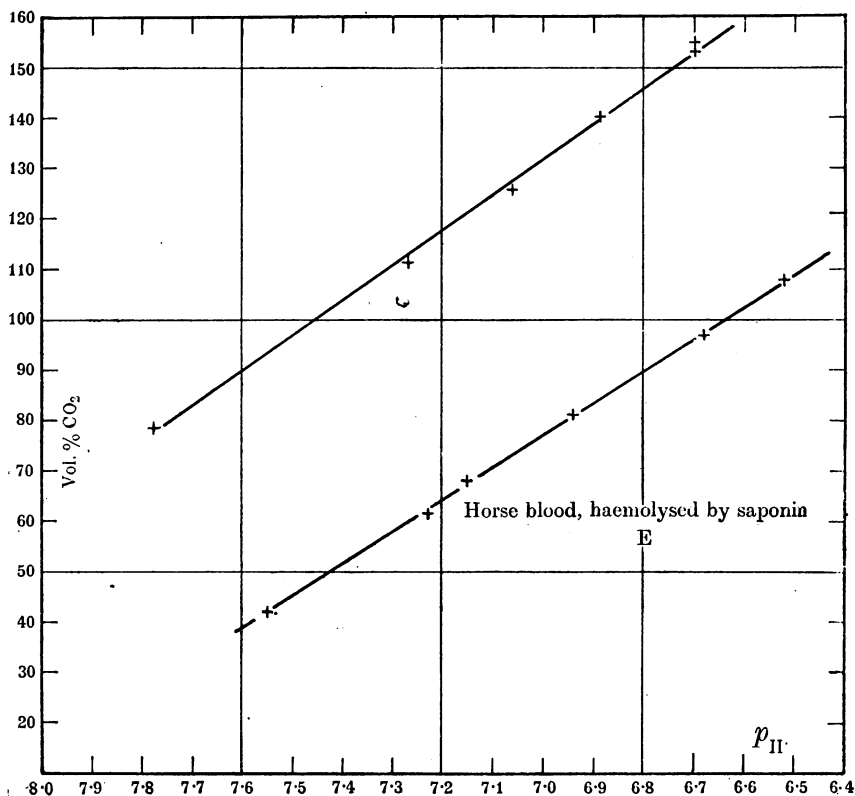


Fig. 25.

In conclusion I will report some experiments carried out in a similar manner with histidine hydrochloride with which Prof. Henriques kindly supplied me, which make it probable the CO_2 combination curve of histidine

can be shifted in a p_{H} -vols. % CO_2 diagram in a similar way to that of blood. The preparation was impure as it only contained 17.1 % N_2 while the theoretical amount for histidine mono-hydrochloride is 26.5 % and for histidine dihydrochloride 21.5 %. A 5 % solution was 0.439*n* as regards chlorine which corresponds to a mixture of mono- and di-hydrochloride. It can however be concluded from the course of the CO_2 combination curve that chloride must also have been present in some other form than histidine hydrochloride. The solutions were prepared in such a way that for the lower curve a 5 % solution of histidine hydrochloride was mixed with an equal part of a sodium bicarbonate solution which combined with 995.9 vols. % just before each experiment, while for the upper curve a bicarbonate solution which combined with 1106.5 vols. % was used. The theoretical distance between the curves should be 55.3 vols. %. The experiments will be found in Table LXI and in Fig. 26. It will be observed the curves are very nearly parallel with an intervening distance of 53.5 to 57.5 vols. % which may be regarded as sufficiently accurate for curves of this type. It should be remarked however that the distance between the curves increases a little with increasing apparent hydrogen ion activity and that the distance in the most alkaline reactions is about 2 vols. % less than the theoretically smallest possible distance so that there must either be small experimental errors or the theory proposed must be incomplete in one way or another.

Table LXI. 2.5 % histidine hydrochloride $p_{\text{K}'_1} = 6.300$ at 18°
and 6.305 at 17° . $Y = 8$.

Curve I.				Curve II.			
Temp.	mm. Hg CO_2	Vols. % CO_2 combined	p_{H} calculated	Temp.	mm. Hg CO_2	Vols. % CO_2 combined	p_{H} calculated
17°	22.5	35.7	7.41	18°	23.5	76.1	7.72
"	40.5	44.3	7.24	"	48.8	86.8	7.46
"	83.4	57.7	7.05	"	113.2	101.6	7.17
"	188.5	78.7	6.83	"	207.7	119.0	6.97
"	392.3	103.0	6.63	"	390.4	141.7	6.77
"	731.0	127.5	6.45	"	730.0	167.5	6.50
Theoretical distance 55.3 vols. %.							

In the above discussion an assumption has been made which is not really tenable, namely that a complex combination between the CO_2 and proteins or histidine does not drive CO_2 out of the bicarbonate. If such a combination, for example a carbamino-acid, is in itself an acid it will in virtue of its cations be able to drive out CO_2 . Equation (176) should therefore—if this possibility be allowed—have the form

$$B = (C_1 - C_{\sqrt{f}}(a_{\text{H}^+}) - f_1(P_{\text{CO}_2}) - f_2(P_{\text{CO}_2}, a_{\text{H}^+})) 2226,$$

and the above experiment would be explained without the assumption of an experimental error. Further experiment can alone determine whether this explanation accords with fact but the above experiment proves at any rate that only very small amounts of carbamino-acid can be formed at the reactions investigated.

As the final result of the investigations reported in chapters VIII, IX, X and XI it is clear that the CO_2 of the blood is exclusively or almost exclusively combined as bicarbonate and that the variable amount combined is due to the presence in the blood of electrolytes the dissociation of which varies with the reaction. Such electrolytes are chiefly proteins. On account of the relatively small concentration of the serum proteins their effect will be subordinate to that of the proteins of the blood corpuscles. Haemoglobin is the most active of the blood corpuscle proteins judging from experiments of Setschenow [1879], Bohr [1905], Hasselbalch [1916, 2] and Campbell and Poulton [1920]. It is impossible to estimate the activity of haemoglobin in this respect but it is not improbable that the other proteins and lecithin (Setschenow) are also active. To a limited extent the phosphates will also function in a similar manner.

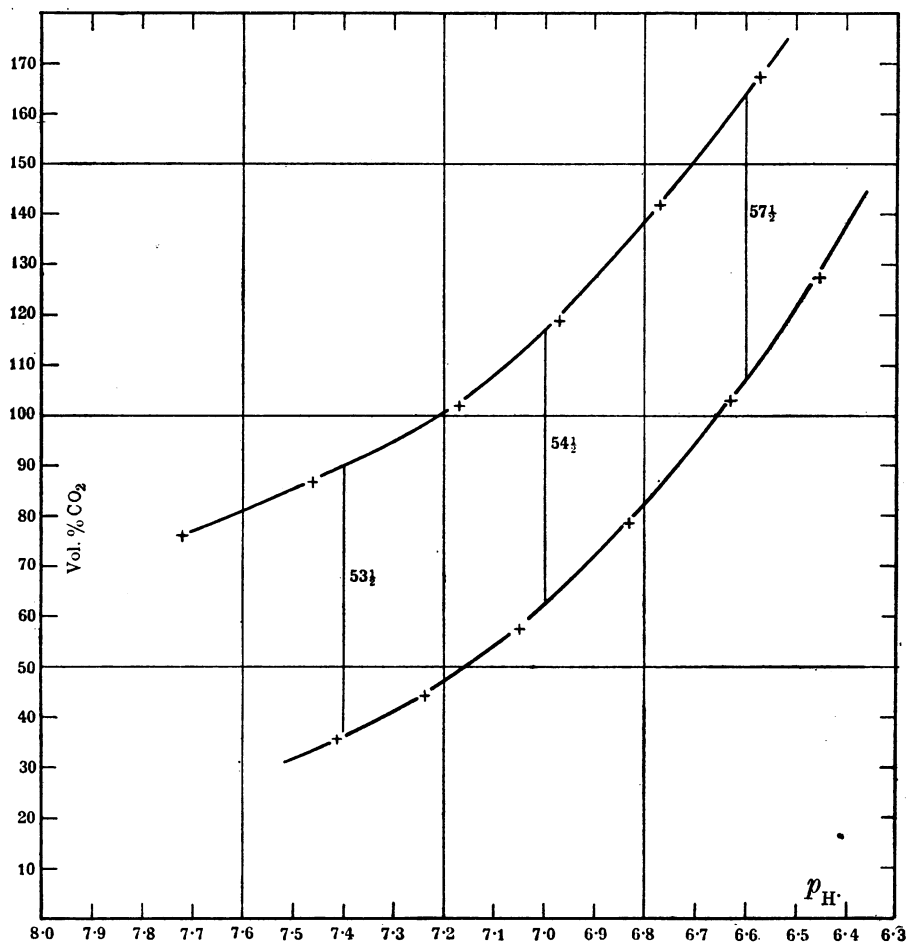


Fig. 26. Histidine hydrochloride. Theoretical distance 55.3 vols. %.

RÉSUMÉ.

I. The theory of the combination of CO_2 in a solution which contains both electrolytes the dissociation of which does and does not vary with the reaction is elaborated.

II. This theory has been tested on phosphate solutions and found to be satisfactory.

III. An empirical relation on a rational basis has been proposed for the combination of CO_2 in serum, haemoglobin solutions and blood.

IV. It has been shown it is only possible for a trifling amount of CO_2 to be adsorbed or bound in a complex manner (as carbamino-acid) in blood or haemolysate.

V. A similar proof has been produced for the combination of CO_2 in a histidine solution.

CHAPTER XII

THE FACTORS WHICH DETERMINE THE PARTITION OF PERMEATING IONS BETWEEN THE BLOOD CORPUSCLES AND THE SERUM, THE VOLUME OF THE BLOOD CORPUSCLES DEPENDENT UPON THE REACTION, AS WELL AS THE POTENTIAL ON THEIR SURFACES

As repeatedly stated A. Schmidt [1867] and N. Zuntz [1867] discovered independently of one another that the amount of alkali in serum increased when the blood was treated with high tensions of CO_2 . Zuntz explained this phenomenon by assuming that the effect of CO_2 was to split off sodium from the sodium-protein compounds of the blood cells and that some of the sodium diffused out of the blood cells in the form of sodium bicarbonate until the sodium bicarbonate concentration was the same in blood cells and serum. When we remember the state of physical, and especially physiological, chemistry at that time the absolute genius displayed in Zuntz's hypothesis excites the greatest admiration.

The converse of this bicarbonate diffusion was discovered in 1874 without its importance in this connection being noticed. Hermann Nasse¹, who at that time was an old investigator and who had gained a considerable reputation in haematology, discovered in 1874 that the chlorine in serum decreased when blood was treated with high CO_2 tensions. At the same time he found that the blood cells swelled under CO_2 treatment, taking up water from the serum. Although he again reported his discovery in *Pflüger's Archiv* in 1878 his name has quite disappeared from textbooks on physico-chemical biology because the honour of discovering the phenomena in question was falsely ascribed to Hamburger [1892, 1902] and v. Limbeck [1894]. In the literature

¹ Do not confuse with the son O. Nasse, the discoverer of isotonic salt solution, or with the father, the celebrated clinician of Bonn, Christian Friedrich Nasse.