At 40 mm. we find in a similar blood 50 vols. % total CO_2 and consequently 47·3 vols. % combined CO_2, from which, in a similar manner to the previous example, we get

		Warburg	
Hasselbalch	Parsons and Donegan	(126)	(130) and correction
$p_{H'(s)} = 7{\cdot}374$	7·421	7·397	7·418

and for blood at 300 mm. CO_2 with 90 vols. % total CO_2 and 70·1 vols. % combined CO_2

		Warburg	
Hasselbalch	Parsons and Donegan	(126)	(130) and correction
$p_{H'(s)} = 6{\cdot}719$	6·787	6·763	6·750

CHAPTER VII

THE REACTION OF THE BLOOD CORPUSCLES.

Although the majority of workers who have investigated the reaction of the blood have realised that what is generally called "the hydrogen ion concentration of the blood" is really only "the hydrogen ion concentration of the serum," few have attempted to get some knowledge of the reaction in the interior of the blood corpuscles, a question however which is of the greatest interest because analogies with other cells may be drawn from it if similar factors determine the difference in reaction between serum and blood cells, and serum and tissue cells. Hasselbalch and Lundsgaard [1912] and later J. M. de Corral y Garcia [1914] claim to have shown that blood cells at physiological CO_2 tensions are more acid than the corresponding serum. The evidence produced is however faulty, as the phenomenon discovered by Hasselbalch and Lundsgaard is only an expression of the Schmidt-Zuntz effect as it appears in A. Schmidt's [1867] and N. Zuntz's [1867, 1868] and many other old experiments, and as is very clearly seen in Hasselbalch and Warburg's [1918] experiments and expositions. Hasselbalch and Lundsgaard showed that the reaction at constant CO_2 tension was more acid in blood than in the serum centrifuged from it. The experiments of the above mentioned authors show that the content of the serum in bicarbonate and therefore the reaction of the serum at a given CO_2 tension is a function of the CO_2 tension in the blood at the time of centrifuging, so that we cannot conclude anything about the reaction in the interior of the blood cells from a difference of reaction between serum in blood and separated serum.

The Schmidt-Zuntz phenomenon, which has been the cause of Hasselbalch and Lundsgaard's error, indicates that some kind of equilibrium prevails between the activity of the ions in blood cells and serum but it is not possible at the moment to say how this equilibrium is maintained. In the final chapter an attempt will be made to give a theoretical and experimentally workable solution of the problem on a relatively broad basis.

If the reaction of blood is determined electrically and then haemolysis produced (*e.g.* by freezing) without letting the blood come in contact with any new gas mixture, the reaction after haemolysis will show whether there is any difference of reaction between blood corpuscles and serum. The assumption involved in this reasoning—as for numerous other instances later in the chapter—is that the dissociation of the electrolytes determined by the reaction is the same at the same reaction before and after the haemolysis.

Konikoff [1913] has reported experiments in which he estimated the reaction of blood electrometrically using Hasselbalch's method. He employed a special electrode vessel with a relatively large platinum plate and assumed he avoided the oxygen error in this way (demonstration of the oxygen error was the real object of his work). Having determined the reaction of the blood he haemolysed it by freezing and found that the reaction had become much more acid.

Milroy [1917] determined the reaction in haemolysed blood at a CO_2 tension of about 40 mm. and found a p_H of roughly 6·60, that is about ten times as large a hydrogen ion activity as in blood serum at the same CO_2 tension.

Although Konikoff devised a special technique to avoid the O_2 error (he did not however make use of minimal immersion) and Milroy employed Höber's principle and was aware of the existence of the O_2 error, I do not hesitate to say that the results of both these investigators are misleading as they undoubtedly had considerable quantities of O_2 in their haemoglobin solutions. Neither of them attempted to prove that the oxygen was actually dissipated during the measurements and it is beyond all question that too low potentials will be obtained if the estimations are carried out with deeply immersed platinum electrodes in strongly oxygenated haemoglobin solutions.

Parsons [1917] has published some electrical determinations in haemolysed blood in which the reaction was almost the same as that usually met with in serum with a similar CO_2 tension.

L. E. Walbum [1914, p. 231] has shown that the reaction in a solution of blood corpuscles (10 blood + 90 physiological NaCl) is the same before and after haemolysis. Although the quantity of blood corpuscles in the experiments was rather small this is counterbalanced by the liquid containing them (serum + NaCl solution) being relatively poor in buffer substances. L. S. Fridericia [1920], and J. Joffe and E. P. Poulton [1920], in the papers extensively referred to in a previous chaper, calculated the reaction in blood corpuscles and serum at the same CO_2 tension in a manner which in essence is identical with that employed in the experiments about to be described, but they made the assumption that $p\lambda_{(c)}$ was the same as p_{K_I} in bicarbonate solutions of the same carbonic acid binding power, an assumption which is to a certain extent supported by Hasselbalch's [1916, 2] rather scanty estimations in dialysed haemoglobin solutions.

It is therefore hardly possible from experiments in the literature to con-

clude anything with certainty about the reaction in the blood corpuscles but I believe that Joffe and Poulton's contribution must be looked upon as the most important on this subject even though it is open to objection as $p\lambda_{(c)}$ was not experimentally determined.

If (120) $$a_{H^{\cdot}(c)} = \lambda_{(c)} \frac{100 - Q(1-D)}{100D} \times \frac{P_{CO_2}\alpha}{7{\cdot}60B}$$

is divided by (119) $$a_{H^{\cdot}(s)} = \lambda_{(s)} \frac{100 - Q(1-D)}{100} \times \frac{P_{CO_2}\alpha}{7{\cdot}60B}$$

we get $$\frac{a_{H^{\cdot}(c)}}{a_{H^{\cdot}(s)}} = \frac{\lambda_{(c)}}{D\lambda_{(s)}}, \quad \text{.....(132)}$$

which in logarithmic form becomes

$$p_{H^{\cdot}(s)} - p_{H^{\cdot}(c)} = p\lambda_{(s)} - p\lambda_{(c)} - \log D. \quad \text{.....(133)}$$

Since we have previously determined $p\lambda_{(s)}$ and D, we only require the value $p\lambda_{(c)}$ for estimating the difference in reaction between blood corpuscles and serum, which we will now attempt to determine. The determination of $p\lambda_{(c)}$ was associated with much greater difficulties than I originally expected. One of the most important was to get rid of the oxygen at the reactions and temperatures dealt with, but this was overcome to a large extent by the technique described in chapter IV. It was easy to haemolyse ox blood by repeatedly freezing so that it became completely transparent and only a trifling amount of blood corpuscles was left, but it was practically impossible by freezing alone to haemolyse horse blood so thoroughly. Even after freezing and thawing three times numerous intact blood corpuscles are present and many amorphous fragments are seen with the microscope. If the volume of the disperse phase is determined by the haematocrite—which can easily be done—it will never be found to be over 5 % of the whole system even when very concentrated blood cell suspensions are used. If a liberal amount of saponin is added to horse blood it will become completely transparent and only a few formed constituents (about 1 % in the haematocrite) can be seen with the microscope. This difficulty of haemolysing horse blood by freezing led me to work with blood haemolysed by saponin as it was found that the combined CO_2 was the same whichever of the two methods was employed as the following experiment shows.

Defibrinated horse blood was concentrated by centrifuging. Haematocrite reading 59·5.

mm. Hg CO_2	Vols. % combined CO_2	
24·3	45·2	Saponin
24·0	45·0	Freezing
79·2	72·7	,,
79·9	72·9	Saponin

In using a concentrated solution of horse blood haemolysed by saponin a new difficulty arose. When it is treated for a long time with high tensions of CO_2 it becomes very viscous and shortly afterwards a large quantity of haemoglobin crystals separate out so that the experiment has to be abandoned. It has been found that this precipitation never takes place in the first quarter

of an hour so that a preliminary treatment of the haemoglobin solution with CO_2 may be undertaken for this space of time and the experiment continued with lower CO_2 tensions (lower $a_{H^\cdot}$). This crystallising out of horse haemoglobin will be reverted to in chapter XI.

In horse blood haemolysed by freezing (concentrated in the centrifuge) I have only once seen a similar crystallisation, and the haemoglobin solution was in this instance cooled to 0°.

That haemoglobin very readily crystallises out at high CO_2 tensions has been known a long time and is mentioned for example by Preyer [1871] without any particular comment. I. Setschenow [1879, p. 48] reported similar observations with strong concentrated frozen horse blood at room temperature (CO_2 and H_2SO_4 addition).

As already repeatedly mentioned the potential in an electrical determination of reaction falls when the platinum electrode has been in contact with protein solutions for some time. The drop is not large and in the course of 2–3 hours an almost constant potential seems to be reached (within $\frac{1}{2}$ millivolt), but I tried nevertheless to avoid any possible error from this cause ("deterioration" of the electrode) by developing the technique employed with L II described earlier in this work. In using this electrode vessel, in which it will be remembered the platinum electrode does not come in contact with the haemoglobin before the potentiometry is started, the potential was found to rise quickly about 10–20 millivolts in the first quarter of an hour after contact (total immersion) was established but quite irregularly. Then it became constant for a time and afterwards slowly declined. When the platinum was heated to redness before platinising the rise was much less, but as a rule a few millivolts. This must be what Parsons [1917] referred to when he wrote that it is essential to heat the electrode red hot before every determination in haemolysed blood.

In Table XXII $p\lambda_{(m)}$ is calculated from Hasselbalch's experiments with dialysed haemoglobin in weak sodium bicarbonate solution the conversion being carried out in the same way as in the preceding chapters.

$p\lambda_{(m)}$ is in agreement with $p\lambda_{(s)}$ and $p\lambda_{(c)}$ and therefore in haemolysed blood we have

$$p\lambda_{(m)} = p_{H^\cdot} + \log \frac{P_{CO_2}\,\alpha}{7{\cdot}60} - \log \beta, \quad \text{.....(134)}$$

where β is the mean concentration of combined CO_2 (expressed in vols. % CO_2) in the haemolysed blood.

In Table XXIII[1] a number of determinations of $p\lambda_{(m)}$ in haemolysed ox blood are given. They were done with the small saturation electrode and within the same period as the experiments with blood designated series A in chapter V.

[1] The temperature corrections here and in what follows are made by adding 0·0075 to the value found for each degree over 18°, and subtracting the same amount for each degree under 18°. In the calculations from experiments in chapters V and VI 0·005 was used as the correction, but the difference is so small that I have not found it necessary to recalculate these earlier experiments with the correction employed in this chapter.

It is extremely probable that the apparent hydrogen ion exponent at this time was 0·06 too low, according to which $p\lambda_{(m)}$ in haemolysed ox blood with about 33 vols. % combined O_2 should be about 6·27.

The determinations in Table XXIV of haemolysed ox blood with 29 vols. % O_2 at 20° and 38° give respectively values of 6·26 and 6·15. They belong to series B and are carried out with the small saturation electrode.

The experiments in Table XXV were made in the large saturation electrode L I. In almost all cases a preliminary treatment with CO_2 for $\frac{1}{4}$–$\frac{1}{2}$ an hour was undertaken. Analyses of two gas mixtures are given in the table corresponding to one measurement of potential, the first relating to the last gas in the spirometer and the second to the gas in the electrode vessel.

$p\lambda_{(m)}$ at 18° referring here to horse blood cells haemolysed by saponin with about 38 vols. % combined O_2 is found to be 6·32; for ox blood haemolysed by freezing with 35 vols. % O_2 it is 6·26.

In Table XXVI measurements are given carried out with L II without heating the platinum electrode red hot before platinising. The results are quite in agreement with those reported in Table XXV, as $p\lambda_{(m)}$ in horse serum haemolysed blood cells with about 40 vols. % combined O_2 is also 6·32, while in haemolysed ox blood with about 32 vols. % it is 6·27. It is worth noticing that the final potential is not appreciably different although the platinum plate with one technique was 2–2½ hours in contact with the protein and with the other technique only $\frac{1}{4}$–$\frac{1}{2}$ hour. I should however expect that with a sufficiently extensive series of measurements a deviation of 1–2 millivolts might be demonstrated.

In Table XXVII are given measurements carried out in L II with platinum plates freshly heated to redness. The results are as will be seen a little different from those just obtained, $p\lambda_{(m)}$ in haemolysed horse blood and ox blood of similar constitution being respectively 6·35 and 6·28 at 18°. These values differ but slightly from those of the first series and the difference is hardly greater than the experimental error, a calculation of the mean error as in the case of the experiments with serum being hardly feasible.

It will be remembered that $p\lambda_{(s)}$ is 6·29 (see chapter VI) and since $p\lambda_{(m)}$ in a mixture of one part serum and three parts haemolysed blood corpuscles is 6·35 we shall not make a large error by assuming $p\lambda_{(c)} - p\lambda_{(s)}$ is 0·07, but as this difference is rather uncertain calculations have been made using (133) with values ranging from 0·05 to 0·09. The results are to be found in Table XXVIII. In the last column the values for (132) are given, the difference here being 0·07.

Table XXII. $p\lambda_{(c)}$ in dialysed haemoglobin with $NaHCO_3$ 0·025n calculated from K. A. Hasselbalch's experiment at 38°.

mm. Hg CO_2	Vols. % combined CO_2	p_H corrected	$p\lambda_{(m)}$
20·2	42·2	7·59	7·13
94·2	55·4	7·06	7·15
7·0	32·9	7·95	7·14

Table XXIII. $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids (freezing) determined by the small saturator electrode. Series A.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'}$	$p\lambda_{(m)}$ 18°	
19·5	459·9	0·5	117·3	0·6	6·51	6·19	1st day
19·0	77·7	0·5	70·5	0·7	7·09	6·21	
19·0	73·7	0·4	69·8	0·7	7·12	6·23	Ox blood
18·0	50·2	0·9	60·6	—	7·21	6·21	2nd day
18·0	50·2	0·9	63·0	0·8	7·19	6·18	Preliminary treatment with CO_2
18·0	28·6	0·5	50·2	1·8	7·36	6·18	
19·5	28·6	0·5	49·5	0·6	7·35	6·20	„ „
			Colorimetric 32				
19·0	37·4	0·5	61·1	0·7	7·33	6·20	1st day
19·5	57·1	0·5	68·6	1·4	7·23	6·23	
19·5	57·1	0·5	70·2	0·5	7·19	6·19	Preliminary treatment with CO_2
19·0	131·9	0·5	90·3	1·6	6·97	6·22	Ox blood
18·0	575·7	0·5	136·2	0·3	6·49	6·20	2nd day
19·0	82·6	0·5	79·1	0·8	7·12	6·22	
18·5	82·6	0·5	82·7	0·7	7·13	6·22	Preliminary treatment with CO_2
18·0	14·3	0·5	40·7	1·3	7·58	6·21	
					Mean	6·21	
20·0	38·6	147·0	51·9	32·2	—	—	3rd day
20·0	314·4	320·8	103·2	34·0	—	—	
19·0	103·5	0·5	77·4	1·1	7·08	6·29	1st day
19·0	40·3	0·2	—	—	7·33	—	
19·0	46·7	0·3	64·5	—	7·28	6·22	Horse blood
19·0	481·5	0·0	128·2	0·9	6·58	6·24	2nd day
			Colorimetric *circ.* 32				

Table XXIV. Washed ox blood cells haemolysed by freezing *circ.* 225 cc. + 6 cc. $n/3$ Na_2CO_3. Determinations of $p\lambda_{(m)}$ in the small saturator electrode. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H'}$ (measured)	$p\lambda_{(m)}$ 20°	$p\lambda_{(m)}$ 38°	$p_{H'}$ (calculated)
27. ii. 19								
38	589·2	0·9	80·8	0·4	—	—	—	6·42
20	615·8	1·0	101·5	0·7	—	—	—	6·41
38	114·3	0·3	47·2	0·6	6·90	—	6·15	6·90
20	119·5	0·3	67·2	0·5	6·95	6·26	—	6·95
38	64·7	0·5	37·3	0·2	7·05	—	6·15	7·05
20	64·6	0·5	55·4	0·7	7·15	6·27	—	7·14
28. ii. 19								
38	121·7	0·6	49·1	0·5	6·88	—	6·13	6·90
21	127·2	0·6	69·2	0·4	6·93	6·25	—	6·93
38	12·1	0·5	13·7	0·7	7·32	—	6·14	7·33
20	12·1	0·5	27·4	0·4	7·53	6·24	—	7·55
38	41·0	0·4	29·8	—	7·09	—	6·09	7·15
20	47·4	0·4	49·3	0·8	7·21	6·26	—	7·21
						Mean 6·26	6·15	
1. iii. 19								
38	36·2	140·0	22·8	28·4	—	—	—	7·10
20	37·8	146·5	38·6	29·3	—	—	—	7·20
38	112·6	565·3	42·2	29·0	—	—	—	6·87
20	117·6	590·6	62·8	—	—	—	—	6·92
38	501·3	199·1	78·4	27·2	—	—	—	6·39

Table XXV. $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids estimated with L I. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\lambda_{(m)}$ 18°		
—	46·9	0·5	—	—	—	—		
19·5	46·5	0·6	64·4	0·3	7·39	6·33	1st day	
—	106·2	0·7	—	—	—	—		
20·5	105·4	2·3	86·5	1·9	7·13	6·30	2nd day	Horse blood haemolysed with saponin
—	29·4	0·8	—	—	—	—		
19·0	29·4	0·9	50·4	0·9	7·48	6·33	3rd day	
			Colorimetric 32					
—	40·2	0·5	—	—	—	—		
20·0	40·4	1·2	64·7	1·2	7·48	6·35	1st day	Horse blood haemolysed with saponin
—	16·6	0·2	—	—	—	—		
19·0	15·3	1·2	40·7	2·1	7·65	6·31	2nd day	
—	12·3	0·5	—	—	—	—		
18·0	12·9	1·1	38·4	1·3	7·67	6·28	3rd day	
			Air from blower	37·6	Mean	6·32		
—	48·4	0·2	—	—	—	—		
19·0	47·7	0·7	66·7	?	7·40	6·34		Human blood, frozen
—	586·5	0·2	—	—	—	—		
18·0	586·5	0·4	144·9	0·2	6·57	6·27	1st day	Concentrated by centrifuging
—	60·8	0·5	—	—	—	—		
18·0	66·4	0·5	85·3	0·2	7·28	6·26		Ox blood, frozen
—	27·2	0·2	—	—	—	—		
18·0	26·7	0·7	64·1	0·2	7·55	6·26	2nd day	
			Air from blower	35·1	Mean	6·26		

Table XXVI. Determinations of $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids made with L II. The electrode was not heated to redness before each measurement. Series B.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	p_H	$p\lambda_{(m)}$ 18°		
—	47·0	0·1	—	—	—	—		
21·0	47·0	2·9	60·7	1·4	7·35	6·31	1st day	
—	258·3	0·1	—	—	—	—		
20·0	258·8	0·2	113·0	—	6·89	6·33		Horse blood, frozen
—	74·2	0·1	—	—	—	—		
19·5	73·7	1·6	75·9	0·5	7·23	6·30	2nd day	
—	45·6	0·1	—	—	—	—		
19·0	45·2	1·2	62·1	0·5	7·40	6·33		
18·0	334·7	0·3	—	—	—	—		
—	335·2	0·3	122·1	0·1	6·78	6·31		
			Air from blower	39·7	Mean	6·32		
20·5	24·6	5·8	43·4	3·3	7·47	6·30	1st day	
—	32·2	0·3	—	—	—	—		
20·0	31·6	3·8	48·8	2·1	7·38	6·27	2nd day	Ox blood, frozen
—	101·3	0·6	—	—	—	—		
20·0	95·0	0·8	74·6	0·4	7·06	6·23	3rd day	
—	305·2	0·1	—	—	—	—		
19·0	305·2	0·7	104·4	0·3	6·73	6·28		
			Air from blower	31·8	Mean	6·27		
			Haematocrite number	72·8				

Table XXVII. Determinations of $p\lambda_{(m)}$ in mixtures of serum and blood cell fluids made with L II. Freshly burnt out platinum electrode.

Temp.	mm. Hg CO_2	mm. Hg O_2	Vols. % combined CO_2	Vols. % combined O_2	$p_{H^\cdot}$	$p\lambda_{(m)}$ 18°		
—	33·7	0·0	—	—	—	—		
19·0	33·3	1·9	53·0	1·5	7·50	6·38	1st day	
—	375·3	0·1	—	—	—	—		
19·0	375·0	0·1	117·6	0·1	6·73	6·32	3rd day	Horse blood, frozen
—	20·1	0·1	—	—	—	—		
19·0	23·6	0·6	54·5	0·0	7·63	6·33	4th day	
—	60·3	0·0	—	—	—	—		
19·0	61·6	0·0	75·1	0·0	7·36	6·37		
		Air from blower		36·3				
—	29·6	0·1	—	—	—	—		
16·0	29·7	0·1	57·5	0·0	7·50	6·31		Horse blood, frozen
		Air from blower		41·6				
—	18·9	0·3	—	—	—	—		
16·0	18·9	0·7	44·3	1·4	7·57	6·30		Horse blood, frozen
—	343·1	1·0	—	—	—	—		
18·5	342·1	2·4	120·7	1·2	6·85	6·39		Horse blood, frozen
—	89·9	0·2	—	—	—	—		
18·5	90·5	1·7	80·8	0·0	7·25	6·39		
		Air from blower		38·2	Mean	6·35		
—	388·9	0·1	—	—	—	—		
17·0	388·4	1·3	110·8	0·6	6·70	6·34		
—	22·8	0·2	—	—	—	—		
16·0	22·6	2·4	51·3	1·2	7·51	6·25		Ox blood, frozen
—	78·4	0·4	—	—	—	—		
16·0	78·9	0·2	76·3	0·1	7·17	6·28		
—	215·1	0·2	—	—	—	—		
16·0	214·5	0·6	99·8	0·1	6·82	6·25		
		Air from blower		27·8	Mean	6·28		

Table XXVIII. The difference of reaction between blood cells and serum (horse blood at room temperature).

$p\lambda_{(s)} - p\lambda_{(c)}$		−0·05	−0·06	−0·07	−0·08	−0·09	−0·07
		$p_{H^\cdot(s)} - p_{H^\cdot(c)}$					$\frac{a_{H^\cdot(c)}}{a_{H^\cdot(s)}}$
$p_{H^\cdot(s)}$	$-\log D$						
6·50	*+0·055*	+0·005	−0·005	−0·015	−0·025	−0·035	0·966
6·88	*+0·097*	+0·047	+0·037	+0·027	+0·017	+0·007	1·06
7·27	*+0·155*	+0·105	+0·095	+0·085	+0·075	+0·065	1·22
7·40	*+0·180*	+0·130	+0·120	+0·110	+0·100	+0·090	1·29
7·60	*+0·222*	+0·172	+0·162	+0·152	+0·142	+0·130	1·42
7·90	*+0·301*	+0·251	+0·241	+0·231	+0·221	+0·211	1·70

A dotted line is drawn through the table which indicates the reactions at which blood cells and serum have the same reaction. It will be seen this is the case between 6·50 and 6·88. Fig. 11 is a graphic representation of the table, the apparent hydrogen ion exponents of serum being the abscissae and the differences between the exponents of serum and blood cells the ordinates. For clearness only the curves relating to $p\lambda_{(c)} - p\lambda_{(s)}$ 0·05, 0·07 and 0·09 are given.

It will be further seen from the tables and curves that the apparent hydrogen ion activity is larger in blood corpuscles than in serum at serum reactions more alkaline than $p_{H^{\cdot}} = 6{\cdot}9$ ($a_{H^{\cdot}} = 1{\cdot}26 \times 10^{-7}$) and that the difference increases with the hydrogen ion exponent.

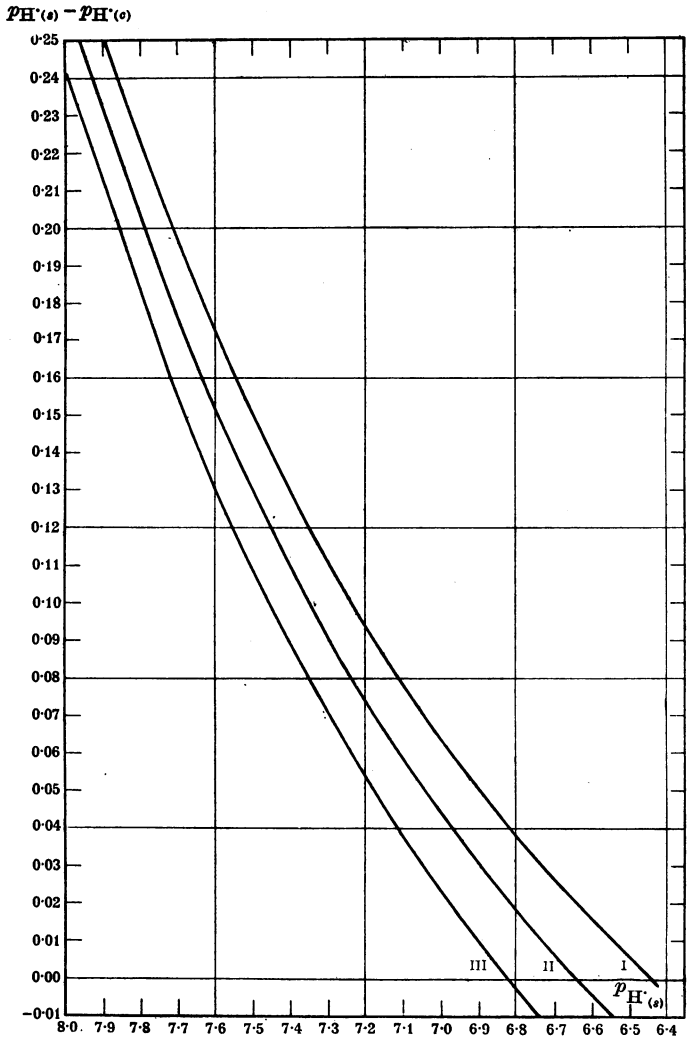

Fig. 11. $p_{H^{\cdot}(s)} - p_{H^{\cdot}(c)}$.

As D in human blood at 38° is the same as D in horse blood at 18° the above considerations will also apply to this species of blood if $p\lambda_{(c)} - p\lambda_{(s)}$ is the same. In the tables there is only one measurement of human blood at room temperature to be found but this fits in quite well in the horse blood series. The question can however only finally be settled by many estimations.

According to Fr. Kraus' [1898] experiments D for ox blood appears to be 1·00 at $p_{H^{\cdot}}$ about 6·8. When this is compared with the determinations of $p\lambda_{(m)}$ in ox blood the same reaction should exist in serum and blood cells at this reaction because $p\lambda_{(c)}$ and $p\lambda_{(s)}$ are very nearly identical in such blood.

It appears also from the few determinations of D for ox blood which are reported in a preceding chapter that the difference of reaction between blood cells and serum increases with the $p_{H'}$.

If we assume that the dissociation of electrolytes which varies with the reaction is the same at a similar reaction before and after haemolysis we can draw conclusions from the combination of CO_2 at the same CO_2 tension, before and after haemolysis, about a possible difference of reaction between blood cells and serum. The combination of CO_2 increases with the apparent hydrogen ion activity in serum and in the fluids of the blood cells but it increases most in the latter case. If the same $p_{H'}$ persists after haemolysis as there was in serum and blood cells before haemolysis (*e.g.* in horse blood at $p_{H'}$ 7·60) then the CO_2 combination is not altered. If however there was a higher $a_{H'}$ in blood cells than in serum before haemolysis, $a_{H'}$ will be midway between the original reaction of the serum and blood cells under the given conditions after haemolysis and less CO_2 will be combined with the electrolytes varying with the reaction in blood cells but more with those in serum. The result will be that, altogether, less CO_2 will be combined after haemolysis than before if the volumes of blood cells and serum are equal.

I have only made a few experiments (about ten) with ox blood with this object in view but they all go to show that less CO_2 is combined after haemolysis than before, at alkaline reactions. At reactions about 6·30 the combined CO_2 was almost or actually the same in blood before and after haemolysis. In some experiments in which the osmolar concentration of the blood was a little diminished the difference was smaller than in ordinary blood. In an isolated experiment where the osmolar concentration of the blood was rather increased the opposite was the case. These effects of the changes in the osmolar concentration are in accordance with the theory, as D varies with the variations in volume of the blood corpuscles.

As will be noticed there is a disagreement, although a small one, between the results attained by the first and last mentioned principles for the determination of the difference of reaction in ox blood. From the first principle we concluded that the reaction was identical in serum and corpuscles at $p_{H'}$ 6·8 while the last pointed to the fact that there was no difference as far as $p_{H'}$ 6·3. Further experiments are needed to clear up the matter.

In Table XXIX some of the experiments[1] mentioned are given. Haemo-

[1] The experiment at 38° was particularly interesting as the relation between oxy-haemoglobin and reduced haemoglobin is not altered by haemolysis. This result was supported by several experiments with saponin haemolysed blood which I hope later to have the opportunity of publishing. The phenomenon itself is not without interest because it indicates that the quantity and kind of salt does not play so great a part in determining the form of the O_2 combination curve of haemoglobin as Barcroft [1914] and his collaborators imagined. That the dilution of the haemoglobin due to the haemolysis plays no great part in the relation between oxy-haemoglobin and reduced haemoglobin was only to be expected because they are both diluted to the same degree and there should thus be no change in the extent of oxygenation of the haemoglobin either according to the interpretation of the process expressed by G. Hüffner [1901] in his later papers or by A. V. Hill [1910, 1913, 1921].

If we could estimate the degree of oxygenation with sufficient accuracy we might however expect to find a little greater oxygenation after haemolysis than before at reactions more alkaline than $p_{H'}$ 6·8 because the extent of oxygenation is a function of the reaction; cf. Chr. Bohr, K. A. Hasselbalch and A. Krogh [1904], R. A. Peters [1914, 2], K. A. Hasselbalch [1916, 2] and L. J. Henderson [1920].

Table XXIX.

mm. Hg		Vols. % combined CO_2		Vols. % combined O_2		$p_{H^{\cdot}(c)}$
CO_2	O_2	in blood	in haemoglobin	in blood	in haemoglobin	calculated
		Blood and water-haemolysed blood at 19°				
14·9	151·6	24·1	—	15·0	—	7·25
14·9	151·6	—	21·6	—	14·6	—
53·0	143·3	—	35·8	—	14·4	—
53·0	143·3	37·5	—	—	—	6·98
144·3	163·8	—	47·3	—	14·4	—
144·3	163·8	48·6	—	14·5	—	6·73
164·5	313·2	50·5	—	14·6	—	6·29
164·5	313·2	—	48·5	—	14·7	—
467·6	256·2	—	60·5	—	14·5	—
467·6	256·2	61·6	—	14·3	—	6·32
731·5	trace	—	64·9	—	—	—
731·5	„	67·0	—	1·3	1·4	6·15
		Blood and water-haemolysed blood at 19°				
10·7	153·0	25·9	—	8·0	—	7·60
10·7	153·0	—	24·1	—	8·1	—
34·1	148·1	35·4	—	8·0	—	7·22
34·1	148·1	—	32·9	—	7·9	—
67·3	141·3	40·0	—	—	—	6·99
67·3	141·3	—	38·7	—	8·2	—
135·3	127·0	46·1	—	7·7	—	6·75
135·3	127·0	—	44·8	—	7·4	—
133·0	126·4	45·8	—	—	—	6·76
133·0	126·4	—	44·6	—	—	—
405·9	69·9	54·3	—	—	—	6·33
405·9	69·9	—	54·3	—	—	—
736·0	trace	59·4	—	3·3	—	6·13
736·0	„	—	60·0	—	1·6	—
		Blood and blood haemolysed by freezing at 18°				
17·4	152·1	47·1	—	16·0	—	7·65
17·4	152·1	—	45·1	—	15·6	—
98·4	135·1	—	72·8	—	—	—
96·1	135·6	72·1	—	15·8	—	7·07
430·4	64·8	—	96·5	—	12·9	—
429·2	65·3	96·3	—	14·0	—	6·50
		Blood and water-haemolysed blood at 18°				
37·2	150·4	—	28·0	—	12·9	—
37·2	150·4	30·1	—	12·9	—	7·11
37·2	150·4	—	28·7	—	13·0	—
37·2	150·4	30·2	—	12·8	—	7·11[1]
		[1] CO_2 preliminary treatment for half an hour.				
		Blood and water-haemolysed blood at 38°				
15·3	144·5	—	12·9	—	8·1	—
15·3	144·5	13·4	—	7·8	—	7·25
42·3	138·6	—	19·5	—	7·7	—
42·3	138·6	20·2	—	7·4	—	6·98
114·6	124·2	—	27·0	—	7·1	—
114·6	124·2	27·5	—	7·4	—	6·62
213·7	100·1	32·6	—	6·2	—	6·46
213·7	100·1	—	32·0	—	6·2	—
455·6	48·0	40·1	—	2·3	—	6·23
455·6	48·0	—	41·3	—	2·2	—

lysis was brought about either by freezing or by the addition of water. When haemolysis by water was completed sufficient NaCl was added to make the salt content up to 0·9 % again.

I. Setschenow [1879, p. 44] reported the following experiment at 37°–37°·5. Emulsion of dog blood corpuscles:

514·6 mm. CO_2, total CO_2 in 50·18 cc. = 50·31 cc. (0°, 1 mtr.).

After freezing:

513·3 mm. CO_2, total CO_2 in 50·18 cc. = 49·98 cc. (0°, 1 mtr.).

From this $p_{H'}$ is 6·22 if $\Psi = 15$ and $p\lambda_{(m)} = 6{\cdot}20$, at which reaction there seems to be the same reaction in dog blood cells and serum.

RÉSUMÉ.

The apparent hydrogen ion activity in horse blood corpuscles has been determined.

CHAPTER VIII

THE DETERMINATION OF THE FIRST DISSOCIATION CONSTANT OF CARBONIC ACID AND THE DEVIATION COEFFICIENTS OF THE BICARBONATE ION.

In the foregoing chapters we have determined the value of $p\Lambda_{(s)}$ and $p\Lambda_{(c)}$, and it will now be interesting to inquire into the factors which control these constants rather more closely.

It will be remembered that in chapter III it was shown that the apparent activity coefficient of the continuous phase of serum and of the blood cell fluid participates in the constants and a rather large number of determinations have therefore been performed of the apparent activity coefficients in salt solutions. I have at several points pursued the investigations further than was absolutely necessary for the problem being dealt with, because it may be of particular interest from a purely physico-chemical standpoint, especially since the appearance of Bjerrum's theory.

The first dissociation constant of carbonic acid has been determined by the conductivity method by J. Walker and W. Cormack [1900] and by J. Kendall [1916] on the basis of experiments carried out by himself, by Pfeiffer[1], by Knox[1] and by Walker and Cormack. The determinations of Walker and Cormack and Kendall are better than the earlier ones (Pfeiffer's and Knox's) and therefore the values calculated from them are the most valuable. The molecular conductivity of the bicarbonate ion at "infinite dilution" comes into the calculation. This value is obtained by extrapolation from conductivity determinations of sodium bicarbonate solutions (and also calcium bicarbonate solutions), but it seems to the author that Walker and Cormack and Kendall have not executed this extrapolation in a satisfactory

[1] Cited from Kendall [1916].