THE DISSOCIATION CURVE OF BLOOD.

By JOSEPH BARCROFT, M.A., B.Sc., *Fellow of King's College, Cambridge,* and MARIO CAMIS, M.D.

(*From the Physiological Laboratory, Cambridge.*)

I. *Historical.*

The available information concerning the dissociation curves of the blood of different animals under varying conditions has been reviewed so frequently in late years that it is only necessary to refer the reader to such works as those of Bohr, of Loewy and of F. Müller in the text-books of Nagel and Oppenheimer.

From the general mass of information discussed in these articles a few points will be mentioned which will serve as an introduction to our own work.

(1) The dissociation curves of hæmoglobin solutions as determined by different observers, and indeed by the same observer (Hüfner) at different times, are not identical[1]. The general nature of the differences is shown by a consideration of the percentage saturation of hæmoglobin at two specified tensions of oxygen.

	Hüfner 'old curve'	Hüfner 'new curve'	Bohr (2)
Percentage saturation of oxygen at 14 mm. pressure	84	63	35
Percentage saturation of oxygen at 35 mm. pressure	93	80	65

(2) The dissociation curves for blood are no more uniform than those for hæmoglobin solutions. Loewy, for instance, working with human blood found the following values in different experiments:

Approximate tension (in mm.)	27	20	45
Percentage saturation in different experiments	49	41	59
	68	49	86
	—	58	—
	—	65	—

Whilst Zuntz and Loewy, working on dog's blood, arrived at the following, amongst other results:

Approximate tension (in mm.)	15	24	32
Percentage saturation in different experiments	12·5	42	54
	36	43	75
	54	63	—
	—	64	—

(3) Bohr discovered that the CO_2 tension had considerable effect on the percentage saturation of blood with oxygen. Probably some of the divergencies noted above are due to differences in the CO_2 tension.

(4) Even at constant CO_2 tensions the blood of different animals (dog [3] and horse [4]) was found to differ by the workers at Copenhagen, and not only so but it differed very materially from the dissociation curve of hæmoglobin.

(5) To explain the latter difference Bohr [5] suggested that two possible causes, (*a*) the alkalinity of the blood, (*b*) the concentration of the hæmoglobin, might affect the affinity of the hæmoglobin for oxygen, the latter increasing, the former decreasing it.

No experimental details are given to support these suggestions, nor are the differences between the curves obtained from different animals explained.

II. *Hæmoglobin.*

In our earliest experiments we found, as others had done [6], that there was a tendency for methæmoglobin to be formed when a solution of hæmoglobin was shaken at 38 % for some time. This is especially so in the presence of mercury; to avoid it we observed two precautions, (1) we were careful to use no mercury, (2) a trace of ammonium carbonate (1 drop of approximately sat. solution) was added to 3 c.c. of hæmoglobin solution. Working thus and using solutions of hæmoglobin prepared by Hoppe Seyler's method we obtained results as discordant as those of our predecessors and indeed we succeeded in imitating (though not at will) both the older and the more recent curves of Hüfner, as well as getting several intermediate curves. It seemed probable that the discordance in our results was due to the traces of ammonium carbonate added, and we consider that the starting point of our research, in so far as positive results are concerned, took place when we made a solution of dog's hæmoglobin after Bohr's receipt, dissolved the crystals in distilled water, rid the solution so obtained

of ether and determined a series of points as follows from three different solutions:

TABLE I.

Dissociation of hæmoglobin crystals dissolved in distilled water.

Solution I:					
Tension of oxygen (in mm.)	12·5	15·5	31	45	72
Percentage saturation ...	29	40	60	77·5	90·5

Solution:	II	II	II	III
Tension of oxygen (in mm.)	20	26	31·5	39
Percentage saturation ...	45	58	66	68

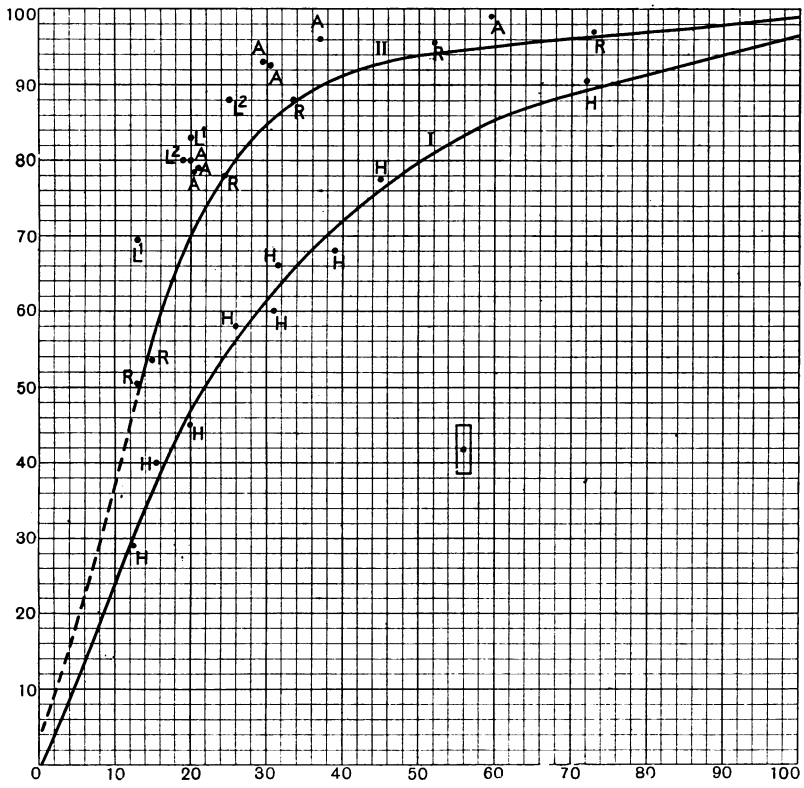

Fig. 1.

I. Bohr's dissociation curve of oxyhæmoglobin dissolved in water.

II. Dissociation curve of hæmoglobin in Ringer's solution. L^1 and L^2 represent different samples of laked blood (ammoniacal). A hæmoglobin solution made alkaline with ammonium carbonate. The rectangle around the point (at 42 % and 56 mm.) indicates the experimental error to which the determinations are liable. Temperature 37—38° C.

Fig. 1 shows the relation of the results tabulated above to Bohr's curve, they fall so nearly upon it that there can be no doubt as to the accuracy of the curve for a solution of hæmoglobin crystals in distilled water.

Such a solution cannot be regarded as chemically pure, it contains small quantities of salt which could only be removed by dialysis, and even when free from ether it has a characteristic odour similar to that of dog's blood.

A comparison of these results with those which we had previously obtained from solutions of hæmoglobin made alkaline with ammonium carbonate as already mentioned, or with blood laked by the addition of dilute ammonia, showed that there was a fundamental difference between the dissociation curves of the hæmoglobin under the different conditions of solution (see Fig. 1).

The following were the data obtained:

TABLE II.

Dissociation of hæmoglobin made alkaline by the addition of 1 *drop of ammonium carbonate to* 3 *c.c. of solution. (Points A in Fig.* 1.)

Tension of oxygen (in mm.)	20·5	21	20	30·5	29·5	37	59·5
Percentage saturation ...	78·5	79	80	92·5	93	96	99

TABLE III.

Dissociation of blood laked by the addition of 1·5 *c.c. of very dilute ammonia to* 1 *c.c. of blood. This ammonia solution was made by the addition of* 2 *c.c. of ammonia of sp. gr.* ·880 *to* 500 *c.c. of distilled water.*

Tension of oxygen (in mm.)	13	19	20	25
Percentage saturation ...	69	80	83·5	88

The divergence between the properties of hæmoglobin in alkaline solution and in distilled water made us anxious to study the properties of the pigment when dissolved in some medium which approximated more closely to a physiological fluid. A solution of hæmoglobin crystals in Ringer's fluid (the analysis of which is given in the Appendix) was made and its properties determined as investigated.

TABLE IV.

Successive points on the dissociation curve of Hb. in Ringer's fluid. The points as actually determined are marked R, Fig. 1.

Abscissa	10	15	20	25	30	40	50	100
Ordinate	36	56	70	79	85	91	94	99

The preceding experiment made it clear that the dissociation curve of hæmoglobin in Ringer's solution occupied a place intermediate between that of an aqueous and of a more strongly alkaline solution of the pigment.

It was not evident, however, whether the altered affinity of the hæmoglobin for oxygen was due to the slight alkalinity of the Ringer's solution, or whether it was a function of the combined effects of all the saline constituents of the fluid. We therefore crystallised some dog's hæmoglobin afresh and divided the crop of crystals into two portions, the first of which we dissolved in ·7 % NaCl and the second in distilled water. With each solution we performed experiments for the purpose of determining the readiness with which the hæmoglobin parted from its oxygen. The results are plotted in Fig. 2, points N^1—N^5.

The aqueous solution of the hæmoglobin was then investigated as a control; the points which were obtained fell on Bohr's line within the errors of experiment, they are shown in Fig. 2 as W^1, W^2, W^3.

From these data, together with a few similar observations subsequently to be described, we can set down the constants of the dissociation curve of hæmoglobin in ·7 % NaCl as follows:

TABLE V.

Ordinates (saturation) and abscissæ (tension) of points on the dissociation curve of oxyhæmoglobin in ·7 % NaCl solution.

Abscissa	10	15	20	25	30	35	40	50	60	100
Ordinate	27·5	41	60	69·5	75	79·5	83	85·5	91	98·5

This curve lies in a position intermediate between that of an aqueous solution of hæmoglobin and of a solution of hæmoglobin in Ringer's fluid.

It had now become evident that the abstract conception of a single dissociation curve of hæmoglobin as such must be abandoned, and that the curve depended not merely upon the reaction but also upon the nature and strength of the saline constituents of the solvent. The comparison of some other base with sodium suggested itself, the most natural for the purpose was potassium.

A fresh stock of hæmoglobin crystals was obtained from dog's blood by the use of Bohr's method. It was divided into three portions. The first was dissolved in ·9 % potassium chloride (equimolecular with ·7 % NaCl); the second and third portions were used as controls and were dissolved in ·7 % NaCl and in distilled water respectively.

The data obtained from the potassium chloride solution are plotted in Fig. 2, points K^{I}—K^{IV}.

Of two determinations with the ·7 % NaCl solution one was at a tension so low that the difference between the NaCl curve and the water curve is not to be distinguished (Fig. 2, N^{VI}), the difference between this point and K^{I} is however very clear. The other N^{VI} takes its place on the sodium chloride curve whilst the aqueous solution yielded a point (W^{VII}, Fig. 2) which takes its place close to Bohr's line and is clearly differentiated in saturation from N^{4} and K^{III}, though within five milli-

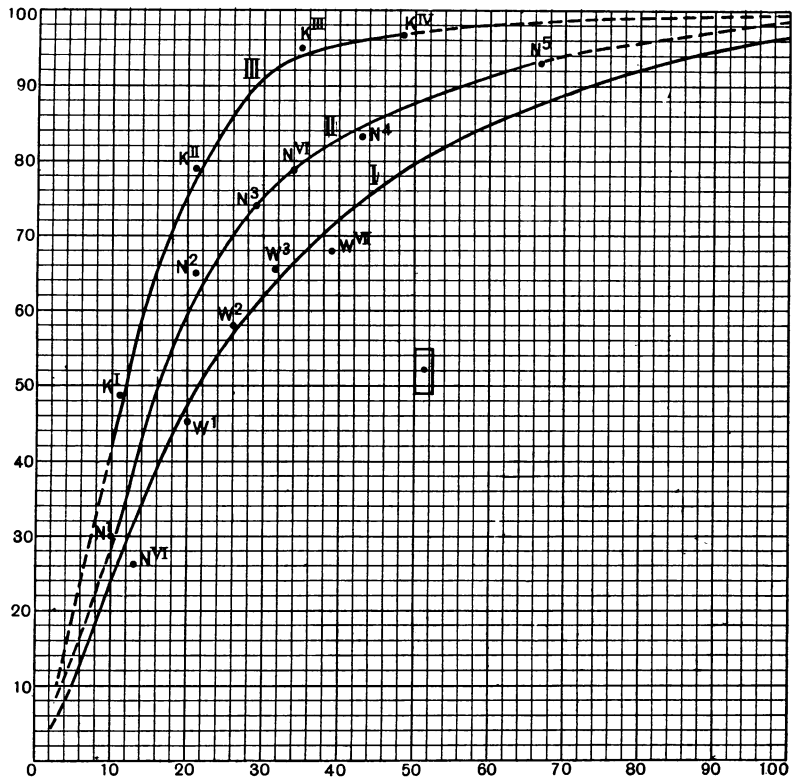

Fig. 2.

Ordinate = percentage saturation of hæmoglobin. Abscissa = tension of oxygen in mm. of mercury.

I. Dissociation curve of hæmoglobin dissolved in water.
II. ,, ,, ,, ,, ·7 % NaCl.
III. ,, ,, ,, ,, ·9 % KCl.

Rectangle surrounding point = magnitude of experimental error. Temperature 37—38° C.

metres of the same tension. The following are the constants for the dissociation curve of oxyhæmoglobin in a ·9 % solution of potassium chloride.

TABLE VI.

Ordinates and abscissæ of points on the dissociation curve of oxyhæmoglobin in a ·9 % KCl solution.

Abscissa	10	15	20	25	30	35	50	100
Ordinate	42·5	63	75·5	84·5	91	94	96	99

The dissociation curve is a function not only of the base however but also of the acid radicle. In this connexion we have investigated the

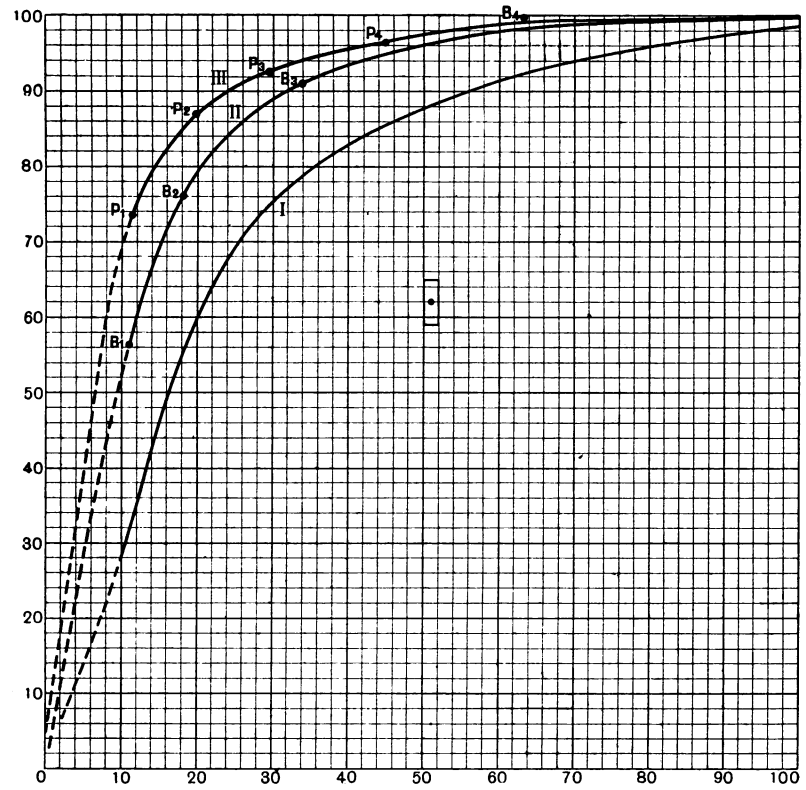

Fig. 3.

Ordinate = percentage saturation of hæmoglobin. Abscissa = tension of O_2 in mm. of mercury. Dissociation curves of hæmoglobin dissolved in

I. ·7 % NaCl, II. $NaHCO_3$, III. Na_2HPO_4,

of strengths equivalent for sodium. Temperature 37—38° C.

properties of solutions of hæmoglobin, not only in sodium chloride, but in sodium bicarbonate and in monacid sodium phosphate.

The sodium bicarbonate was of a strength equivalent in the matter of sodium to ·7 % NaCl, not therefore equimolecular, the same was the case with the Na_2HPO_4.

The data for Na_2HPO_4 are shown in Fig. 3 as points B_1—B_4.

The constants of the curve to which these data approximate are as follows:

TABLE VII.

Ordinates and abscissæ of points on the dissociation curve of a solution of hæmoglobin in 1·01 % *$NaHCO_3$.*

Abscissa	10	15	20	25	30	35	40	50	100
Ordinate	52	69·5	79	85	89	91·5	93·5	96	99·5

The corresponding data for Na_2HPO_4 are shown as points P_1—P_4 in Fig. 3.

TABLE VIII.

Ordinates and abscissæ of points on the dissociation curve of a solution of hæmoglobin in 1·6 % *of Na_2HPO_4.*

Abscissa	10	15	20	25	30	40	100
Ordinate	67·5	81	87	90·5	93	95	99·5

III. *Blood.*

Early in our experiments we were struck with the difference between human blood and that of certain animals, the cat and dog for instance. This difference had been observed by Loewy who compared his results obtained from human blood with those obtained, in conjunction with Zuntz, on dog's blood. In the actual determinations of individual samples which they investigated there was so much overlapping that the difference which appeared on the average was not very convincing, especially as the necessity for working at a specified carbonic acid tension was not then appreciated.

The effect of carbonic acid on the dissociation curve. To clear the ground we set ourselves to verify Bohr's[3] investigation of the effect of carbonic on the dissociation curve of hæmoglobin. This we did, in so far as the general principle was concerned, in the most unequivocal way. In certain questions of detail our results differ from those of

Bohr, these differences perplexed us somewhat at the time and we established them by renewed investigation. In the light of our subsequent work it is clear that they are to be explained, to some extent at all events, by the fact that we worked on sheep's blood, whilst Bohr's curves apply to the blood of the dog—to this extent our results are new and we therefore append them. They possess another slight interest inasmuch as we endeavoured to choose specified tensions of carbonic acid in studying the oxygen dissociation curve of hæmoglobin. This is a laborious process. The amount of carbonic acid which it is necessary to introduce into one of our tubes in order to give a

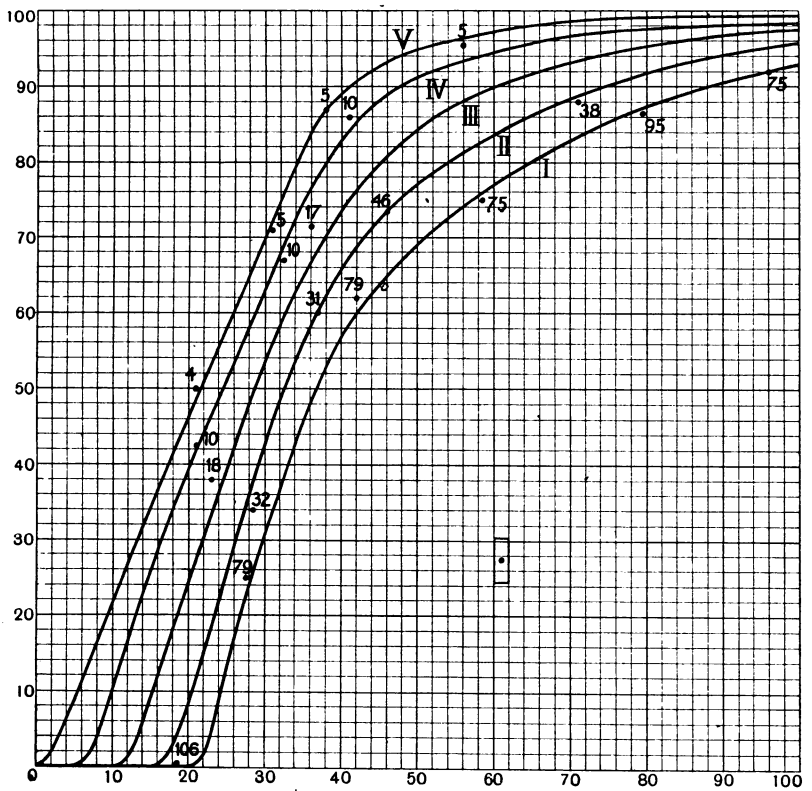

Fig. 4.

Ordinates represent percentage saturation of hæmoglobin, abscissæ tension of O_2 in mm. of mercury. Dissociation curves for sheep's blood at I 5, II 10, III 20, IV 40, and V 80 mm. tensions of CO_2 respectively. The rectangle surrounding the point shows the magnitude of the experimental error to which the observations are liable. The numbers attached to the points show the actual CO_2 tensions at which the observations were made. Temperature 37—38° C.

specified tension is, at the outset, a matter of guess work, since it depends upon unknown factors in the constitution of the blood. At the best one can only expect to be partially successful.

The results which we have obtained are plotted in Fig. 4.

The points marked on the figure as dots represent the actual determinations, whilst the figures attached to them indicate the observed CO_2 tension.

TABLE IX.

Abscissæ and ordinates of points on dissociation curves of sheep's blood at varying CO_2 tensions.

Abscissa (in mm.)	10	15	20	25	30	35	40	45	50	60	70	80	100
Ordinate:													
5 mm. Tension CO_2	28	35	47	58·5	70	81	89	93	95	96	98	99	99·5
10 mm. CO_2	11	26	38·5	51	63	74·5	83	88	91·5	94·5	96·5	97·5	98·5
20 mm. CO_2	0	10	25	49	53·5	65·5	74·5	80·5	84·5	90	93	95	97·5
40 mm. CO_2	0	0	11	26	42·5	56	65	72	77	83·5	88·5	93	95·5
80 mm. CO_2	0	0	1	12·5	31	45·5	56·5	64	69·5	77	83	87·5	92·5

As it seemed uncertain how the carbonic acid acted upon the blood we centrifugalised some dog's blood, the serum was withdrawn, the corpuscles were washed twice in Ringer's solution and divided into two portions. In the first portion the corpuscles were suspended in Ringer's solution in about the concentration of the original blood, whilst hæmoglobin crystals were made of the second and these were dissolved in Ringer's solution. In both cases it was found that CO_2 was inimical to combination with oxygen.

TABLE X.

Effect of CO_2 on corpuscles and hæmoglobin respectively.

	Washed corpuscles.			
Tension of CO_2 (in mm.)	2	5	76	69
Tension of O_2 (in mm.)	18	19	17	18
Percentage saturation	55	57	6	9

	Hæmoglobin in Ringer's solution.							
Tension of CO_2 (in mm.)	0	0	0	0	59	128	117	95
Tension of oxygen (in mm.)	18·5	33	55	78·5	18·5	33	55	78·5
Percentage saturation	66	87	94·5	98	46·5	64	87	91·5

The carbonic acid then tends to turn the oxygen from hæmoglobin, whether the hæmoglobin be contained in the corpuscle or be freely in solution.

In order to study the relation of the dissociation curves of hæmoglobin and of blood it is necessary that each should be investigated at the same tension of carbonic acid.

In the case of hæmoglobin it is simplest to work in the absence of CO_2 or at a negligible tension of say $\frac{1}{2}$—1 mm. This is not easy in the case of blood, and further the lower the CO_2 tension the greater is the effect on the curve of small variations in CO_2. Thus it is apparent from Fig. 4 that there is nearly as much difference between the dissociation curves at 5 and 10 mm. of CO_2 respectively as between 40 and 80 mm. In the case of blood, therefore, it is of advantage to work at a considerable CO_2 tension. In the following comparisons we have endeavoured to keep near to 40 mm. tension of CO_2. This quantity has a certain special interest inasmuch as it is approximately that of the blood in the body.

IV. *Relation of dissociation curves of hæmoglobin and of blood.*

It is not unnatural to suppose that the very existence of corpuscles as such may have some effect upon the dissociation curve of blood. Gases are prone to be liberated from their solutions by minute particles, and *à priori* it is not unlikely that the fact of the hæmoglobin being contained inside a small envelope of great curvature might introduce surface effects which would modify the relations of the gas within and without the corpuscle. We made it our business to ascertain whether any such physical circumstances were responsible for the differences which exist between the dissociation curves of blood and of hæmoglobin.

For any two solutions of hæmoglobin to be directly comparable it is necessary, as we have shown above, to have the hæmoglobin dissolved in the same medium as regards salts, and under the same tension of carbonic acid.

With regard to the salts our task has been rendered easy by the fact that the most excellent analyses of the salts of red corpuscles of many animals have been made within recent years by Abderhalden[8], and these with Schmidt's[8] classical analysis of human corpuscles furnish a very substantial basis on which to work.

The salts in the red corpuscles of different animals differ very much, especially as regards potassium and sodium, the corpuscles of man, pig and rabbit contain much potassium and little sodium, those of the dog and cat contain chiefly sodium, whilst the sheep occupies an intermediate position. Similar differences exist in the case of the acid radicles, in

some corpuscles the phosphates preponderate over the chlorides to a greater degree than in others. In all the analyses the bases appear to preponderate over the acid radicles, the deficiency of the latter being made up by the formation of carbonates.

Our procedure was as follows. Two animal types, man and the dog, were selected, which differed widely from one another in bases contained in their red blood corpuscles. Two portions of the same solution of hæmoglobin in distilled water were taken. To one the salts of the human red blood corpuscle (Schmidt's analysis) were added, to the other those of the dog's red blood corpuscle (Abderhalden's analysis). The way in which the solution was made up was as follows. A graduated measuring cylinder of 25 c.c. was taken; into it was put as much normal HCl as was necessary to provide the calculated quantity of chlorine for 20 c.c. of fluid. This in the case of man is 1 c.c. precisely. To this is added the weighed quantity of phosphoric acid. The analyses are given as P_2O_5, we used metaphosphoric acid in anhydrous sticks, weighing out the quantity which would provide the calculated amount of P_2O_5. To the acid solution in the bottom of the cylinder we added the K and Na in the form of bicarbonate, the excess of CO_2 was shaken off and we were left with about 1 c.c. of fluid containing the required amount of K, Na, Cl, P_2O_5 and HCO_3. Calcium, magnesium and sulphate figure to so small a degree in red blood corpuscles that we determined, in the first instance at all events, to neglect them. Hæmoglobin solution (13 %) in water was then poured into the cylinder till the whole was made up to 20 c.c. In the case of each solution (that which simulated human corpuscles and that which simulated those of the dog) several points on the dissociation curve were determined. The data obtained were as follows:

TABLE XI.

Dissociation of a solution of hæmoglobin in a solution of the salts (K, Na, P_2O_5, Cl and HCO_3). Of the concentration in which they exist in the human corpuscle. (Points H_1—H_4 in Fig. 5.)

Tension of CO_2 (in mm.)	34	41	37	40
Tension of O_2 (in mm.)	16	20	40	73
Percentage saturation	33	44	78·5	94

TABLE XII.

Dissociation of the same solution of hæmoglobin made up with the salts of the dog's red blood corpuscle. (Points H^1—H^4 in Fig. 6.)

Tension of CO_2 (in mm.)	42	43	42	49
Tension of O_2 (in mm.)	21	32	52	68·5
Percentage saturation	23	35·5	73	87·5

For direct comparison with these the dissociation curve of human blood and of dog's blood under similar conditions was investigated with the following results:

TABLE XIII.

Dissociation curve of human blood (defibrinated by whipping) at about 40 mm. Tension CO_2. (Points B_1—B_{12} in Fig. 5.)

Tension of CO_2 (in mm.)	40	45	44	38	38	29·5	37	43·5	45	40·5	31·5	35·5
Tension of oxygen (in mm.)	5·5	8	13·5	16	27	28·5	41·5	45·5	47	57·5	87	159
Percentage saturation	1·5	1·5	9·5	33·5	66·5	62	82	85	83	88	92	97

TABLE XIV.

Dissociation curve of dog's blood (defibrinated by whipping). (Points B^1—B^7 in Fig. 6.)

Tension of CO_2 (in mm.)	37·5	42	40	37	47·5	43·5	39
Tension of oxygen (in mm.)	19	36	41	47·5	58	84	105
Percentage saturation	19·5	43	53	65	76·5	88	91

The direct comparison of the blood, whether human or canine, with the corresponding hæmoglobin solution, as exhibited in Figs. 5 and 6, reveals the fact that the points proper to each animal fall on a single curve which is characteristic of that animal. A hæmoglobin solution made up with the salts of the human red blood corpuscles has a dissociation curve indistinguishable from that of human blood, whilst the same hæmoglobin solution to which are added the salts of dog's red blood corpuscles has the same dissociative properties as dog's blood.

The corpuscle then profoundly influences the relation of the oxygen to the hæmoglobin within it, not by any surface effect, but in virtue of its being a receptacle of certain inorganic salts which are different from those of the medium which surrounds it.

Since the curve given in Fig. 5 represents, as nearly as our present knowledge will permit, the dissociation curve of blood as it circulates in the human body we have determined no less than twelve points on the curve. Previous to some of the determinations we pumped the oxygen from the blood and started our observation on reduced and not on saturated blood. Indeed it is only possible to obtain determinations at points of such low tension as B_1 and B_2 by the addition of this procedure.

The following table shows the relation between the ordinate and

abscissa of various points in the dissociation curves of human and of dog's blood respectively:

TABLE XV.

Blood at approximately 40 *mm. tension of* CO_2 *and* 37—39° *C.*

Abscissa	10	15	20	25	30	35	40	45	50	60	80	100	160
Ordinate for human blood	0	21·5	45·5	59·5	69	75·5	80	83·5	86	88·5	91·5	93·5	97
Ordinate for dog's blood	5	11·5	18·5	20·5	32·5	41·5	50·5	60·5	69	80	88·5	90	—

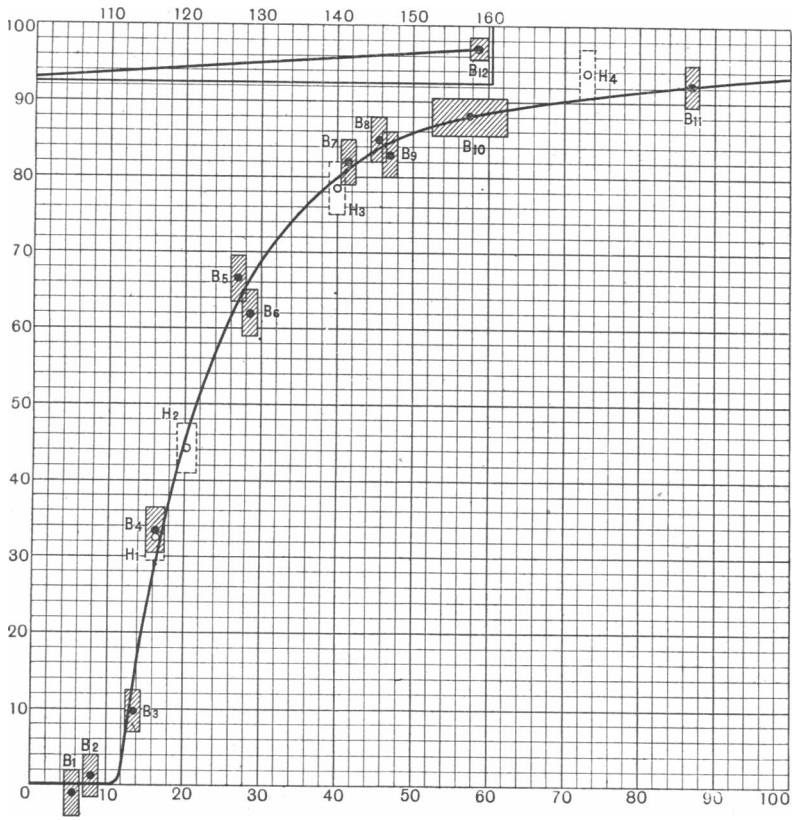

Fig. 5.

Ordinate = percentage saturation. Abscissa = tension of O_2 in mm. of mercury. Dissociation curve of human blood at 40 mm. tension of CO_2. • = determination for human blood. o = ditto for hæmoglobin solution with salts of human red blood corpuscles. The area drawn round the point is the experimental error in each case. Temperature 37—38° C.

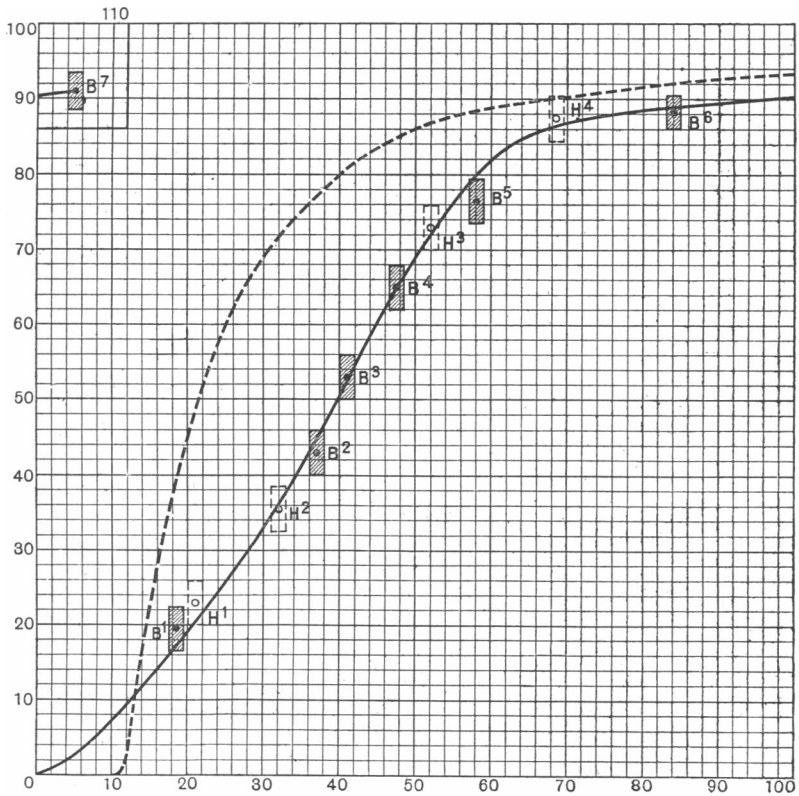

Fig. 6.

Ordinate = percentage saturation of hæmoglobin. Abscissa = tension of O_2 in mm. of mercury. Dissociation curve of dog's blood at 40 mm. tension CO_2. Dotted line = dissociation curve of human blood (see Fig. 5). • = determination for dog's blood. o = ditto for hæmoglobin solution with salts of dog's red blood corpuscle. The area drawn round the point is the experimental error in each case. Temperature 37—38° C.

V. *Some theoretical points.*

1. The dissociation curves of the human blood and of sheep's blood are remarkable for the completeness with which carbonic acid eliminates the oxygen at low oxygen tensions. Thus with 40 mm. tension of CO_2 the amount of oxygen in blood is immeasurably small at oxygen tensions of 15 mm. and under. The value of blood as a medium for respiration depends upon (1) the readiness with which it will take oxygen up in the lungs and (2) the readiness with which it will dispose of it to the tissues. In both of these respects, but especially in the

latter, blood has the advantage over a hæmoglobin solution. In this connexion it is instructive to compare Fig. 5 with Fig. 1. The curve of blood as it exists in the body crosses the curve of a simple hæmoglobin solution at a tension of about 20 mm. of oxygen, above this tension blood, owing to the presence of salts in the corpuscle, chiefly of potassium salts, has a much greater attraction for oxygen than a hæmoglobin solution would have, at 30 mm. oxygen tension for instance the hæmoglobin solution is 62 % saturated, whilst the blood is 69 % saturated, on the other hand at 15 mm. the hæmoglobin solution retains 36 % of its oxygen whilst the blood is practically deoxydised. There seems then to be a very good reason why the respiratory processes of the body are so adjusted as to keep up an adequate tension of carbonic acid in the blood. The point of intersection of the two curves must depend upon the conditions prevailing both in the lungs and in the tissues. At great heights, for instance, the taking up of oxygen by the lungs becomes more difficult, it is reasonable then that the blood curve should shift somewhat to the left in order that the oxygen may be taken up more readily by the lungs, and that a portion of this burden of disadvantage should be thrown upon the tissues, which means ultimately that their activity would be restricted.

That this is what actually occurs at high altitudes has been shown by Ward[9] whose alveolar CO_2 tension fell from 37·7 mm. in London to 28·5 on Monte Rosa. The tuning of the respiratory centre to an abnormally low CO_2 tension at high altitudes aids pulmonary respiration in at least two ways, firstly it increases the amount of oxygen in the alveolar air and secondly it increases the affinity of the alveolar blood for oxygen.

2. The limiting pressure of oxygen in the alveolar air is stated to be 30 mm. approximately. This fact presents itself with much more force in the light of the curves which have been determined by Bohr and by ourselves than in those determined by Hüfner. The tension of 30 mm. means but 69 % saturation in human blood. Now the numerous observations of Hill and Nabarro[10] indicate that in the dog muscle depletes the blood of 70—80 % of its normal quotum of oxygen. Zuntz[11] found an even greater absorption. If this figure is true for man it is clear that blood containing less than 70 % of its usual quantity of oxygen and circulating at the usual rate will fail to satisfy the muscle. Therefore if the muscle is not to suffer from deficiency of oxygen the circulation must quicken and the muscle must either receive blood at the expense of other organs or extra work

must fall upon the heart. We may push our enquiry somewhat further and say a word about each of these alternatives. The heart, itself a muscle, is at the same initial disadvantage as the rest of the musculature of the body and therefore can only ward off its own asphyxia at even its normal rate of working by increasing its blood supply, to require increased work of it is therefore to set up a vicious circle. As regards other organs we need only mention two, the kidney and the brain. These are probably even more sensitive to want of oxygen than muscle. Normally the blood leaving the kidney[12] contains more than 60 % of its oxygen. The following figures show that the blood usually emerges from the kidney containing oxygen at a higher tension, and in three out of four cases cited at a much higher tension than that of the oxygen in the arterial blood which would enter the kidney under conditions which would allow the blood to be only 69 % saturated with oxygen (assuming that the quantities of oxygen in the blood involved are the same for man as for the dog).

Oxygen in arterial blood in c.c. per 100 of blood	Oxygen in venous blood in c.c. per 100 of blood	Percentage sat. of venous blood (art. blood taken to 97 % saturated)	Tension of oxygen in venous blood (human curve) in mm.
23·2	21·7	91	77
13·4	9·7	70	31
17·8	16·9	92	80
24·2	23·6	94	112

As regards the central nervous system we have less information, since the only analyses which exist are of blood from the Torcula and which therefore comes from the meninges and other places as well as the substance of the brain itself. The data of Hill and Nabarro[10] show however that this blood, like that of the kidney, is very arterial. If the blood went to the brain 69 % saturated, and the quantity of oxygen were taken out which was observed by these authors (about 20 % of the whole oxygen capacity) the blood would leave the brain 49 % saturated. The oxygen tensions of the blood entering and emerging from the brain would then be 30 and 21 mm. of mercury respectively. Normally the blood leaves the brain about 80 % saturated, corresponding to a tension of 40 mm. of mercury. Haldane and Boycott[13] have found that an oxygen tension of 60 mm. of mercury in the alveolar air affects the respiratory centre under normal conditions. Apart from the question of secretory activity of the lungs this would correspond to 88 % saturation of the arterial blood and 68 % saturation (tension 29 mm.) of the venous blood (assuming that the blood-flow does not quicken), *i.e.* the venous blood would leave the respiratory

centre at a tension which is approximately that of the oxygen in the arterial at an altitude at which life becomes impossible.

In all these cases the tension of oxygen in the venous blood might be and probably is raised by local vascular dilatation. It is hardly necessary to point out how great would be the strain on the vascular system of endeavouring to maintain the blood-pressure.

3. In what has just been said we have assumed that the arterial blood is in equilibrium with the alveolar air and therefore we have ignored for the moment the secretory theory of respiration. This theory rests chiefly on the ærotonometric work of Bohr, the theory of the invasion and evasion coefficients put forward by the same author, and the determinations of Haldane and Lorrain Smith[14] of the oxygen tension in the alveolar air by the carbon monoxide method. It is clear that the considerations put forward in this paper must affect the interpretation of Haldane and Lorrain Smith's figures to a greater or less extent. Probably it would be more satisfactory to redetermine the relative saturations of blood with oxygen and CO in the presence of CO_2 than to attempt to recalculate their results from the data at present at our disposal.

4. Whilst our experimental determination of the dissociation curve of hæmoglobin agrees precisely with that of Bohr, this very agreement forms a criticism of Bohr's[15] theoretical handling of his data, for he, in opposition to Hüfner, maintains that the concentration of the solution should influence the form of the curve, it should be pointed out very clearly therefore that whilst Bohr's curve was obtained with a six per cent. solution of hæmoglobin our curves were obtained with twelve to fifteen per cent. solutions as determined from our oxygen readings and checked in most cases by a standard Haldane's hæmoglobinometer.

The practical advantage of solutions of this strength, which may be obtained by shaking the crystals with water a little at a time at 38° for about three hours, are very great, inasmuch as the correction necessary for the oxygen physically dissolved in the fluid is correspondingly small.

5. The formula published recently by Wolfgang Ostwald as representing the dissociation curve of hæmoglobin, $\frac{x}{a} = k \cdot c^m$, when x = the quantity of gas absorbed, or in his phraseology "adsorbed," a the quantity of blood (or presumably of hæmoglobin), c the pressure of gas, and k and m constants, is one in which the concentration of the hæmoglobin does not influence the actual form of the curve and is to this extent consistent with our experimental results.

APPENDIX.

The methods employed.

The determinations just discussed have been obtained by a method which is much more rapid than those of previous workers and it involves the use of only small quantities of blood or hæmoglobin solution. Therefore it has become possible to undertake investigations of the dissociation of hæmoglobin under various circumstances on a scale which was quite impracticable with the ærotonometer and the blood gas-pump.

The ærotonometer in the present case is reduced to a simple glass tube of about the size of a test-tube.

This tube was of the pattern described by Bohr for the purpose of collecting gases from the blood gas-pump. It consists of a glass tube open at one end, of length 18 cm. and diameter about 1·3 cm.; at the other end is a three-way "tail-tap," the bore of this and of the tubing immediately connected with it is about 2mm.

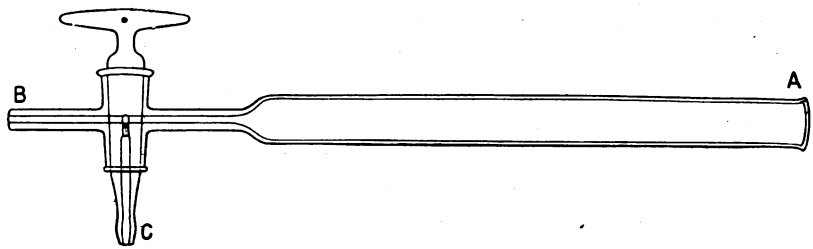

Fig. 7.

Into this is placed $2\frac{1}{2}$ c.c. of hæmoglobin solution, it is then inverted so that it stands vertically, the end *A* being immersed in mercury. The air is sucked out from the end *B* and a piece of rubber tubing which leads from the nitrogen bottle is attached to *B*. The tube contains now only mercury and hæmoglobin solution, a known quantity of air may be introduced at the end *A* over mercury from a measure which consists of a test-tube of the required size. Nitrogen is then introduced at *B*. The tap of the nitrogen cylinder is opened. The gas is allowed to go to waste through *C* until all air which may have been in the connexions is swept out. The tap is then cautiously turned so that the nitrogen goes into the ærotonometer tube. It is allowed to fill the tube until the mercury-hæmoglobin surface has sunk to within a few

millimetres of the end *A* of the tube. The tube is then corked with a rubber cork and inverted so that *B* is downwards. Any mercury which is in the tube is allowed to run out. It is well that the cork should be driven a good way into the tube; when the mercury has run out the pressure inside the tube will still be slightly in excess of that of the atmosphere. The tap is therefore given a rapid turn (with the tube horizontal) so that the two pressures may be equal. The experiment is therefore commenced with the gas at the atmospheric pressure. The ærotonometer tube is placed in the bath at 37—38° and shaken as before. At the end the pressure will have changed for several reasons, (1) the gas is heated, (2) there will be increased tension of aqueous vapour, (3) there will be an exchange of gas between the hæmoglobin and the blood. The volume of the gas is of course constant throughout. These causes of variation in the pressure are of course all calculable quantities, and the third has been left out of account however except in cases where the exchange produced an appreciable alteration of pressure—for instance where a known but considerable quantity of carbonic acid was introduced and subsequent gas analysis showed that the blood or hæmoglobin solution absorbed quantities of the order of 1 c.c. which made a difference of about 5 % to the pressure. In such cases this factor was taken into account.

In the ordinary determinations the following example will show that the inclusion of this factor is unnecessary. Consider a 10 % hæmoglobin solution at approximately 10 mm. pressure of oxygen. If it were saturated when introduced in the apparatus it would be about half saturated at the end, that is to say 2·5 c.c. of it would yield up approximately ·2 c.c. of oxygen. The volume of the tube is about 20 c.c., therefore there would be an error of 1 % in the total pressure of gas in the tube at the end of the experiment. Now the oxygen tension is approximately 10 mm. and is in error 1 % and therefore ·1 mm. But the method professes at most to be exact to 1 mm.

Again suppose the oxygen pressure to be 50 mm. the saturation being about 80 %, 2½ c.c. of blood will give up ·8 c.c. The error caused by this will be ·4 % or ·2 mm. in 50 mm.

When the tube had been sufficiently shaken in the bath it was removed, held vertically with the cork downwards, gripped for a moment with a cold wet towel, care being taken not to shake the tube, the cork was then removed from the tube under mercury and as quickly as possible the necessary fluid for analysis was taken out with a special pipette.

This was of the form shown in Fig. 8. The curved portion, *A*, was of tubing of about one millimetre bore as also was the portion *C*. The length of *C* must be sufficient to allow the end to reach the bottom of one of the blood gas bottles of the differential blood gas apparatus. The major portion of the pipette is one cubic centimetre in capacity between two marks and is made of tubing of about 2 mm. bore. The curved end is immersed in the mercury and slipped into the eudiometer tube. Care must be taken to see that the curved portion fills itself with mercury, otherwise a direct air passage is formed into the eudiometer and air may be sucked in.

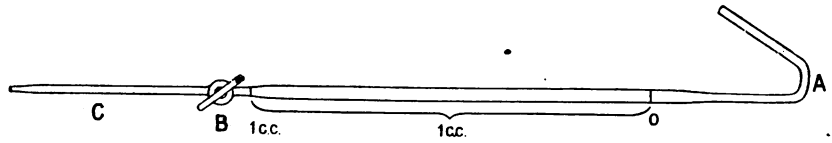

Fig. 8.

Blood is withdrawn from the eudiometer till the blood surface reaches the tap *B*. The tube is then removed and inverted. Any mercury which is in it falls to the region of the tap. When the tap is opened therefore a globule of mercury first flows down the portion *C*. This is followed by blood. Fluid is allowed to run out until the upper meniscus reaches the zero mark. The tube is now practically a burette. The extremity of which is placed in the blood gas bottle. It should touch the bottom of the bottle. In the bottle is $1\frac{1}{2}$ c.c. of ammonia. The blood is therefore delivered gently and lies in a layer underneath the ammonia solution.

When the hæmoglobin solution or blood, as the case may be, has been taken out of the tube the upper end *B* (Fig. 7) may be connected to a Haldane's gas analysis apparatus and a sample of the gas withdrawn and analysed, usually the analysis of the gas was postponed till after the analyses of the fluid had all been made. Under such circumstances the corks were replaced in the tubes and the tubes put back in the warm bath.

The analysis of the gases has been performed in that form of apparatus which was described by Haldane for the analysis of air. The gas burette was of 10 c.c. capacity, the bulb 7 c.c. in size and the stem was divided in hundredths of a c.c. The samples of gas used by us for analysis were usually of about 8 c.c. Taking this quantity a hundredth of a c.c. of oxygen corresponded to a tension of about 1 mm.

of mercury. As there was no difficulty in reading the meniscus to half a division, *i.e.* to 1/200 c.c., the apparatus was sufficiently accurate for our purpose.

In our earliest observations we made duplicate analysis of the gas in the tube. This however we found later to be an unnecessary complication.

The analysis of the blood gases. The blood has been analysed in the differential method. The apparatus used is in all essentials that described by one of us in this *Journal*, but one or two alterations in detail have been introduced.

(1) Egg-shaped bottles have been used by us in some of our apparatus. This provides for the ammonia lying in a thicker layer over the blood than was the case with the cylindrical or conical bottles.

(2) The arrangement for raising and lowering the fluid in the manometers has been modified.

The percentage saturation of oxygen is found in the following way. Suppose A and B to be the two bottles of the apparatus. 1·5 c.c. of NH_3 is put in each and in A is put 1 c.c. of the blood for analysis, in B 1 c.c. of the same blood which however has been saturated with oxygen. The difference between the oxygen in the two samples of blood is then determined. Let this be $d' \times c$, where d' is the observed difference of pressure and c the constant of the apparatus. When the measurement has been made it is necessary to measure the total quantity of oxygen which A now contains. This is done with ferricyanide in the manner described in a former paper. Let this be $D \times c$. The percentage saturation would then be $\frac{d' \times c}{D \times c} \times 100$, but for a slight correction which must be made for the oxygen dissolved in the plasma. $d' \times c$ is the difference between the amount of oxygen taken up in bottle A and in bottle B—the latter is of course zero since the blood in bottle B was saturated previously to the determination—$d' \times c$ therefore becomes the amount of oxygen taken up by blood A. Now this is taken up by both the plasma and the corpuscles. The simplest way of eliminating the quantity of oxygen taken up by the plasma is to find out what pressure in the manometer it represents at different tensions of oxygen and subtract this from the total reading. In the apparatus we have used it corresponds to 2 mm. at a tension of 0 mm. of mercury, 1·5 at 38 mm., 1 at 76 mm., above which tension we have worked but seldom, let this difference of pressure be p then, d the difference of pressure due to the oxygen absorbed by the hæmoglobin of the blood in A is equal to $(d' - p)$.

Since the ferricyanide turns the oxygen from the hæmoglobin only there is no such correction in the division of the fraction, and therefore the corrected percentage saturation

$$S = \frac{100 \times d \times c}{D \times c} = \frac{100\,(d' - p)\,c}{D} = \frac{100\,(d' - p)}{D}.$$

The fact that the constant of the apparatus does not come into the calculations renders the calculations much simpler.

It is important to observe that the ratio of p to D is small. The blood of different animals differs considerably in the quantity of hæmoglobin present. Usually however D varies from about 45 mm. in the cat to about 70 mm. in the dog, p therefore varies from about 4 to about 2 % of D.

In the case of hæmoglobin solutions it might easily be otherwise. Most previous workers have made their observations on hæmoglobin solutions of a concentration of about 5 %. For such a solution D would be about 20 mm. and therefore p would be as much as 10 % of D. It will be obvious therefore that p may become a very considerable quantity relatively to d', for it is only at very low tensions that d' is more than half D. We have therefore endeavoured to obtain as strong hæmoglobin solutions as possible, and for the most part we have been successful in getting solutions which contain as much hæmoglobin as does blood and which frequently gave readings of 110—120 on Haldane's hæmoglobinometer. We obtained solutions by the very gradual addition of water to the crystals which separate from the etherial solution combined with incessant shaking at 38° C.

Analysis of CO_2 used in experiments.

$CO_2 = 98{\cdot}93$ %, $N_2 = 0{\cdot}82$ %, $O_2 = 0{\cdot}25$ %, $CO = 0{\cdot}000$ %.

Analysis of Ringer's Solution used in experiments.

NaCl 6·3 gr., KCl 0·25 gr., $CaCl_2$ 0·45 gr., $NaHCO_3$ 0·15 gr., distilled water one litre.

Accuracy of analyses. The calculated experimental error is shown as a rectangle in each of the Figures 1—6. The meaning of the rectangle is this, that if the point determined (say in Fig. 3) is the point in the middle of the rectangle the dissociation curve would fall within the specified area, there being the possibility that the saturation as measured was wrong to the extent of 3 % which might be in either direction, whilst the tension should not be more than 1 mm. wrong—*i.e.*

the oxygen reading should not be out more than ·01 c.c. in about 8 c.c. of gas; the gas burette read easily to ·005 c.c.

That the observed errors were within the calculated limits are shown by the following observations where duplicate analyses were made.

1. Sheep's blood tension of oxygen 38 mm., duplicate samples of blood were analysed giving saturations of 82 % and 83 % respectively (uncorrected for oxygen in plasma).

2. Sheep's blood tension of oxygen 54·5, saturation 70 and 67·5 % (ditto).

3. Hæmoglobin solution (alkaline); three tubes similarly treated.

		(*a*)	(*b*)	(*c*)
Tension (in mm.) ...	...	20	20·5	21
Percentage saturation	...	80	78·5	79

4. Washed corpuscles (in presence of CO_2); two tubes similarly treated.

		(*a*)	(*b*)
Tension (in mm.) ...	...	18	19
Percentage saturation	...	55	57

5. Washed corpuscles (in absence of CO_2); two tubes similarly treated.

		(*a*)	(*b*)
Tension (in mm.) ...	...	17	18
Percentage saturation	...	6	9

6. Hæmoglobin solution (alkaline); two tubes similarly treated.

		(*a*)	(*b*)
Tension (in mm.) ...	...	29·5	30·5
Percentage saturation	...	93	92·5

SUMMARY.

1. Curves of the dissociation of hæmoglobin in water and in certain saline solutions have been determined.

2. Confirmation has been obtained of the dissociation curves of hæmoglobin in water as observed by Bohr and of the effect of CO_2 in dissociating the oxygen from blood.

3. The dissociation curve of human blood has been determined at 38° and 40 mm. tension of CO_2.

4. This curve is contrasted with that of dog's blood under similar circumstances.

5. The differences between the dissociation curves of human and dog's blood are to be explained by the differences in the saline constituent of the red blood corpuscles of the respective animals.

6. Some theoretical application of the data given in the paper are considered.

(The cost of this research has been largely defrayed by a grant from the Royal Society.)

BIBLIOGRAPHY.

(1) Loewy. Anat. u. Physiol. p. 246. 1904.
(2) Bohr. Zentralbl. f. Physiol. XVII. p. 689. 1904.
(3) Bohr, Hesselbach and Krogh. Skand. Arch. f. Physiol. XVI. p. 409.
(4) Krogh. Skand. Arch. f. Physiol. XVI. p. 400.
(5) Bohr. Zentralbl. f. Physiol. XVII. p. 691. 1904.
(6) Ernst Ziemke and Franz Müller. Arch. f. Anat. u. Physiol. Supp., p. 177. 1901.
(7) Bohr, Hesselbach and Krogh. Skand. Arch. f. Physiol. XVI. p. 412.
(8) Abderhalden. Lehrb. d. physiol. Chem. Zweite Aufl. p. 732 *et seq.*
(9) Ward. This Journal, XXXVI. p. 387. 1908.
(10) Hill and Nabarro. This Journal, XVIII. p. 218. 1895.
(11) Zuntz. Berliner klin. Wchnschr. p. 141. 1878.
(12) Barcroft and Brodie. This Journal, XXXIII. pp. 58–63. 1905–6.
(13) Haldane and Boycott. This Journal, XXXVII. p. 355.
(14) Haldane and Lorrain Smith. This Journal, XXII. p. 231. 1898–9.
(15) Bohr. Zentralbl. f. Physiol. XVII. p. 684.
(16) Barcroft. This Journal, XXXVII. p. 12.