THE LAWS OF COMBINATION OF HÆMOGLOBIN WITH CARBON MONOXIDE AND OXYGEN.

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In a succeeding paper the results will be given of a number of experiments by the carbon monoxide method of determining the oxygen pressure of the arterial blood. As the method is based on a quantitative interpretation of the law of combination of carbon monoxide with hæmoglobin in presence of different partial pressures of oxygen, we found it necessary to re-investigate this law with a view to its more complete elucidation. The results are contained in the present paper, and throw considerable new light on the mode of combination of hæmoglobin with oxygen, as well as with carbon monoxide.

From a number of experiments Haldane and Lorrain Smith[2] concluded that when a solution containing hæmoglobin is saturated with a gas mixture containing oxygen and CO the relative proportions of the hæmoglobin which enter into combination with the two gases are proportional to the relative partial pressures of the two gases, allowing for the fact that the affinity of CO for hæmoglobin is about 300 times greater than that of O_2. The dissociation curve of HbCO in presence of pure air mixed with varying percentages of CO, or of a gas mixture containing a constant percentage of CO and a varying percentage of oxygen, is thus, according to their conclusions, a rectangular hyperbola. Their experiments were nearly all made at about 15° or 37° with dilute solutions of ox-blood, and all were in the absence of CO_2. Only three rather rough experiments were made with undiluted blood.

At that time it was still generally believed that the dissociation curve of the oxyhæmoglobin in blood is a rectangular hyperbola, and

[1] The former two authors are responsible for the experimental results; the latter for the mathematical analysis.

[2] This *Journal*, xxii. p. 231. 1897.

that the curve given by Hüfner in 1891 was at any rate approximately correct. The influence of CO_2 and that of salts on the curve were also quite unsuspected. The researches of Zuntz and Loewy, Bohr, Barcroft, and their associates, have, however, altered completely the current conceptions as to the dissociation of oxyhæmoglobin; and this fact alone rendered a further investigation of Haldane and Lorrain Smith's conclusions very desirable. We also knew (from a series of unpublished experiments made some years ago by one of us) that the experiments of Haldane and Lorrain Smith at 15° were not quantitatively exact, as the saturations had not been quite complete; and Dr Krogh had also kindly informed us in a letter that he had obtained results different from those of Haldane and Lorrain Smith when he used the blood of a different animal[1].

In the present experiments, which were begun in 1909, we have (unless otherwise stated) used undiluted blood at 38°. In order to make certain of obtaining maximum saturation of the blood with the carbon monoxide we have employed the following method.

By means of a pipette of narrow bore ·05 c.c. of the defibrinated blood was introduced into a cylindrical saturating flask of 400 c.c. capacity. With the help of a curved glass rod the drop of blood was then spread in a ring about half a centimetre in width round the inner surface of the saturator, which was then filled with the air or other gas mixture containing CO, closed with a rubber stopper, and rotated slowly in a water-bath at 38° for an hour or more. The blood was thus exposed in a thin layer in constant motion to the gas mixture. To prevent evaporation a thin film of moisture was deposited on the inside of the glass, by blowing some expired air into the flask before the gas mixture was introduced. The gas mixture itself was introduced either by allowing about four litres of it to blow through the flask, or (in experiments on dissociation curves) by first filling the flask with pure hydrogen (free from traces of CO), or air, or any other mixture, and then introducing measured quantities of oxygen, CO_2, and CO through a capillary tube in the stopper by means of pipettes of accurately known capacity, the gas being enclosed between two taps, one at each end of the pipette. After driving in the oxygen and CO_2 by mercury pressure, and mixing, the excess of pressure was allowed to blow off, and the CO (of which the quantity was small) was then introduced by pressure of water, the water being allowed to fill the tube in the stopper; and in case of any small bubble of gas being left behind in the connections the

[1] Published afterwards by Krogh in the *Skand. Arch. f. Physiol.* XXIII. p. 217. 1910.

water was again withdrawn and returned. The CO employed was always analysed, and was usually in the form of a mixture with air. After its introduction the rubber tube connecting the pipette with the capillary tube was closed with a clip, and the flask placed in the water-bath and rotated. After a short time a sample was withdrawn into a Haldane gas analysis apparatus for determination of the oxygen and CO_2, and finally the excess of pressure was blown off in the water-bath, which was kept covered to exclude light. After the rotation had been continued for half an hour or more the motor was stopped and the blood allowed to collect in a drop on the lower side of the saturator. The flask was then removed and opened, and about $2\frac{1}{2}$ c.c. of water were poured in so as to dissolve the drop of blood. The solution was then at once allowed to flow out into a small tube, which was corked up and removed for titration.

To make certain that the saturation was complete we made several control experiments in which blood fully saturated with CO was introduced with the gas mixture into one saturator, and blood containing no CO, but with the same gas mixture, into another. On titration the two samples gave identical results, allowance being made for the CO absorbed or given off by the blood. In most cases an hour is far more than sufficient to effect complete saturation; but the lower the partial pressure of CO and of oxygen in the gas mixture the longer will be the time required; and in working with very low partial pressures we have allowed more than an hour. With human blood, and air containing ·034 % of CO, we found that the maximum saturation with CO had been reached after 15 minutes of rotation, and that with 10 minutes of rotation 85 % of the maximum saturation was reached. It would thus seem that 30 minutes of rotation would give an ample margin in any case where a mixture of ordinary air with CO was used.

In this and the succeeding paper we have not considered it necessary to reproduce all the results of the very numerous gas analyses. It must suffice to say that we found them necessary, and that they were made in every case except where the gas mixture containing CO was pure air. In calculating the partial pressures of the gases we made allowance for the barometric pressure at the time, for the pressure of aqueous vapour, and for the very small quantity of CO absorbed by the blood in the saturator.

The percentage saturation of the hæmoglobin was determined colorimetrically by the carmine method, of which fuller details will be given in the succeeding paper.

In Fig. 1 we have plotted a series of results with air containing various percentages of CO, the blood used being that of J. S. H. and C. G. D. for the upper curves, and that of two mice for the lower ones. In some of the experiments 6 % of CO_2 was added to the mixture of air and CO in the saturating vessel, and in one experiment (not shown in

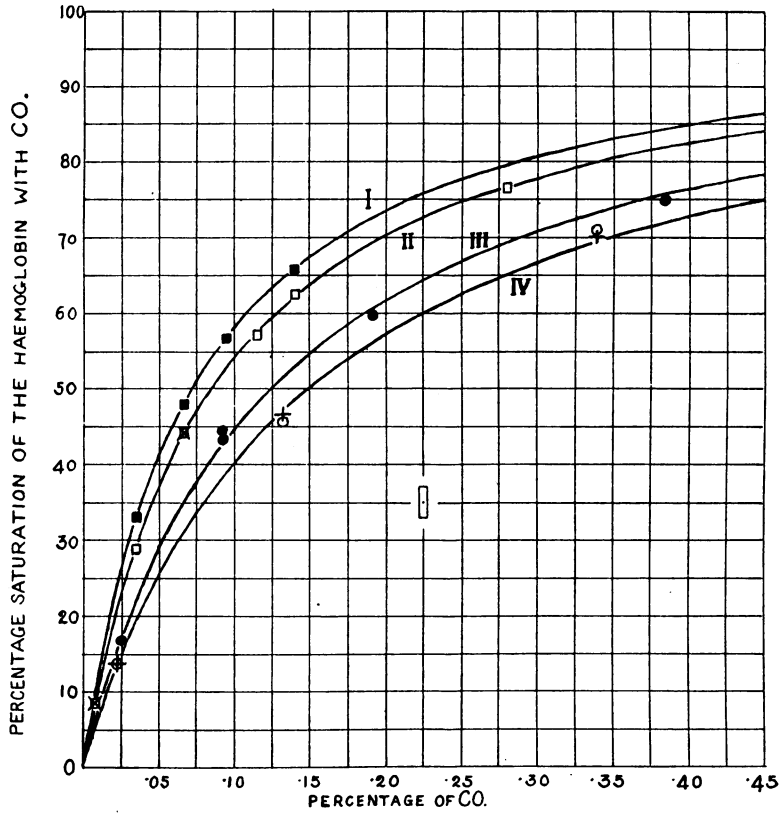

Fig. 1. Dissociation curves of CO-hæmoglobin in presence of air (20·9 % O_2) at temperature of 38°.

I. Blood of J.S.H. II. Blood of C.G.D. III. Blood of mouse *A*. IV. Blood of mouse *B*. The crosses indicate points determined in the presence of 40 mm. pressure of added CO_2.

the Figure, as the result coincided with another result) the blood was first diluted with an equal volume of 1 % solution of dry Na_2CO_3. It will be seen that neither the CO_2 nor the Na_2CO_3 had any appreciable influence on the results. The curves as drawn are rectangular hyperbolas, and all the experimental results lie along these curves within the limits

of error of titration (about 2 % of the total saturation). The figure also shows that although for each individual the curve is a rectangular hyperbola, the percentage saturation with a given percentage of CO varies for different individuals and species. In mice the percentage of CO required to produce a given percentage saturation of the hæmoglobin with CO appears from various experiments, which we need hardly quote

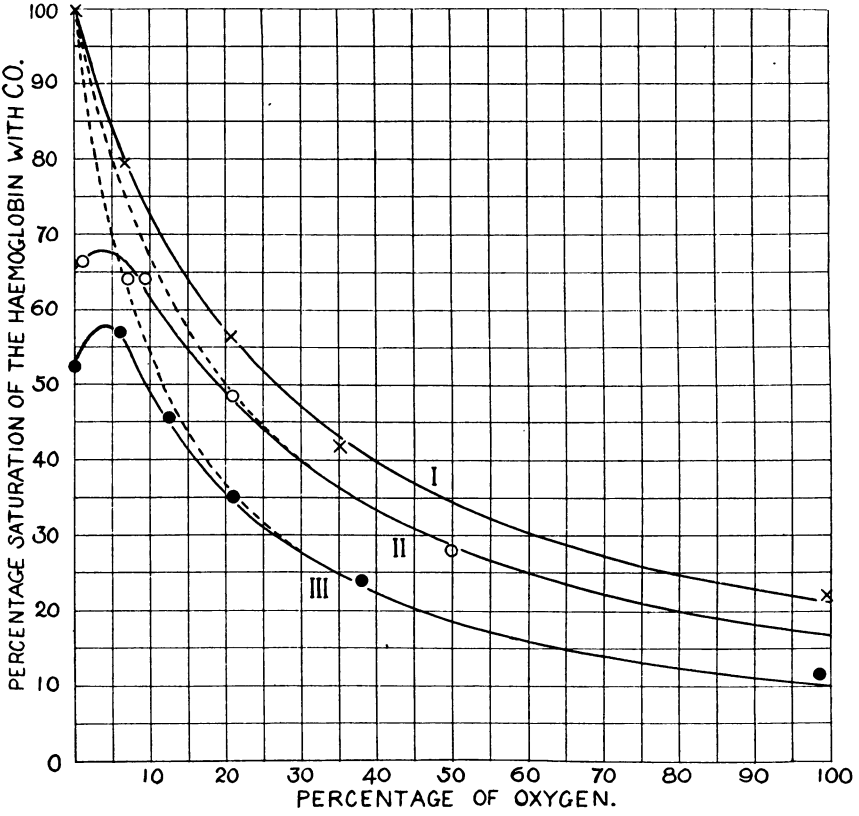

Fig. 2. Dissociation curves of CO-hæmoglobin in presence of constant percentage of CO and varying percentage of oxygen, at atmospheric pressure.

I. Blood of J.S.H. : CO = 0·0945 %. II. Blood of mouse *C* : CO = 0·090 %.
III. Blood of mouse *D* : CO = 0·0635 %.

in detail, to be on an average about 50 % higher than in man. In different samples of mouse blood the percentage of CO required to produce half saturation varied from ·07 to ·15. The results completely confirm Krogh's observation that blood from different animals may give different results, but in other respects are in exact agreement with Haldane and Lorrain Smith's conclusions.

The curves evidently correspond to the simple reversible reaction,

$$HbO_2 + CO \rightleftarrows HbCO + O_2.$$

This correspondence holds good whether the blood is diluted to 1 %, as in Haldane and Lorrain Smith's experiments, or undiluted, as in our own.

Fig. 2 shows the results of two series of experiments in which the percentage of CO was kept constant while the percentage of O_2 was varied. The upper curve is for the blood of J. S. H., and the lower ones for the blood of two different mice. It will be seen that the points determined again lie on the rectangular hyperbolas drawn through them until the dotted portions of the curves are reached. With this exception, therefore, the curves again correspond to the equation given above, and from a single point on the curve for any individual we can construct the whole of the curve, until the dotted portion is reached.

Before discussing the cause of the extraordinary deviation from the curve shown in the case of the mouse blood with very low percentages of oxygen and carbon monoxide we may refer to the influence of temperature and dilution. When blood is saturated with a mixture of air and CO at room temperature (about 15°), instead of at blood temperature, the percentage of CO required to produce a given percentage saturation with CO is very distinctly less. Table I gives some comparative results obtained with the blood of different mice.

TABLE I.

		% saturation of hæmoglobin		Calculated % of CO required to produce 50 % saturation	
	% of CO	At 37°	At 13°	At 37°	At 13°
Mouse No. 1	·0503	32·7	46·0	·104	·059
,, ,, 2	·127	51·6	59·0	·119	·088
,, ,, 3	·127	47·8	56·7	·134	·097
,, ,, 4	·127	57·5	63·7	·094	·074

From this table it will be seen that about 40 % more CO is needed to produce a given saturation at 38° than at 13°. This difference, though considerable, is not to be compared with the effect of temperature on the simple dissociation curve of oxyhæmoglobin, as determined by Barcroft and King[1], who found that a difference of 50 % in the latter curve is produced by a temperature difference of only about 5°. For human blood (of J.S.H.) a number of determinations made some years ago and hitherto unpublished, showed that half-saturation was

[1] This *Journal*, XXXIX. p. 381. 1909.

produced by about ·05 % of CO in air at about 15°, whereas ·072 % is required to produce half-saturation at body temperature. This result corresponds with those for mouse blood.

The effect of dilution with water is somewhat difficult to determine at body temperature, unless the time allowed for saturation is cut rather short, since the diluted hæmoglobin undergoes chemical change rather quickly, this change revealing itself by the fact that on saturation with CO the solution no longer gives the full pink colour characteristic of undecomposed hæmoglobin. At 15°, however, diluted blood appears to give practically the same saturation with a given percentage of CO in air as undiluted blood. It may be noted here that a series of experiments made by one of us some years ago, showed that in Haldane and Lorrain Smith's experiments with diluted ox-blood at room temperatures the saturation produced by gentle shaking for 20 minutes by hand was not quite complete. Violent shaking had to be avoided on account of its effect in producing mechanical coagulation, but satisfactory saturation could easily be obtained by rotating the blood in a saturating bottle by means of a motor for at least half an hour[1]. With the ox-blood used, 50 % saturation of the hæmoglobin was obtained with about ·055 % of CO—practically the same result as with undiluted ox-blood at the same temperature. Addition of a little ammonia, which prevents mechanical coagulation, was found to have no appreciable effect on the curve.

In the following experiment undiluted blood saturated at 38° was compared with blood diluted to 1 % with distilled water at 11°.

	% of CO	Blood undiluted at 38°	Blood diluted to 1 % at 11°
Human blood (J. S. H.)	·093	57·1	67·4
Blood of a mouse	·093	45·2	49·1

From the foregoing it is clear that neither cooling nor dilution nor alteration in reaction abolishes the differences between different kinds of blood in their behaviour towards mixtures of CO and air. The differences appear to be characteristic of the individual men or animals furnishing the blood[2].

[1] The same result can be obtained by violent shaking for five minutes if the blood solution is rendered slightly alkaline by addition of NH_3.

[2] A paper by Hartridge (This *Journal*, XLIV. p. 21, 1912) which appeared while the present paper was passing through the press, furnishes by a new method of determination, independent corroboration of our conclusions as to the effects of temperature, dilution, and changes in reaction. Hartridge also obtained negative results with additions of various salts and of lactic acid.

As CO-hæmoglobin in the complete absence of oxygen has a dissociation curve of its own it is evident that unless the partial pressure of CO is sufficient by itself to produce complete saturation of the hæmoglobin the curves shown in Fig. 2 can never reach 100 % saturation; and as a matter of fact the mouse blood in the lowest curve was only 52 % saturated when the oxygen percentage was reduced to zero. It might, perhaps, have been expected from this latter fact that the curve for the mouse blood would be a simple rectangular hyperbola ending at 52 % saturation. If this were so, however, the curves in Fig. 1 would not be perfect rectangular hyperbolas, as they actually are (at any rate practically speaking), even when the partial pressure of CO is much too low to produce, in the absence of oxygen, more than a merely fractional saturation of the hæmoglobin with CO. Nor would the curves in Fig. 2 follow the course which they actually take. As a matter of fact the course of any curve of the type plotted in Fig. 2 can be predicted up to a certain point without the slightest reference to the dissociation curve of CO in the absence of oxygen. At a certain point, however, when the oxygen percentage becomes very low, the curve begins to deviate, and soon leaves completely the rectangular hyperbola which it had previously followed, to trace out the remarkable hump shown in Fig. 2 for mouse blood. This hump is more and more marked the lower the percentage, or partial pressure, of CO, and is clearly connected with the separate dissociation curves of oxyhæmoglobin and CO-hæmoglobin. The existence of the hump points to the conclusion that the presence of reduced hæmoglobin has a powerful influence of some kind in preventing CO from combining with hæmoglobin; and if so it would be expected to have a similar influence on the combination of O_2 with hæmoglobin. This idea gave a further clue to an understanding of the dissociation curves of oxyhæmoglobin and CO-hæmoglobin; and these curves will be discussed before further consideration of the hump.

As the dissociation curves of oxyhæmoglobin in the blood of C. G. D. and J. S. H. were of considerable interest in connection with other investigations as well as the present one, and we thought at first that the upper part of the curve given by Barcroft for the blood of C. G. D. might possibly be a little too high, we have determined both curves, and the results are plotted in Fig. 3.

The determinations were made with Brodie's modification of the Barcroft-Haldane blood-gas apparatus. About 5 c.c. or more of blood were drawn from a finger, defibrinated, and placed in the saturator of

400 c.c. capacity. The required gas mixture was then introduced. In making this we either used pure hydrogen (wholly free from CO) with oxygen added, or air partially deprived of oxygen by respiration, the CO_2 being absorbed by soda-lime. Sufficient CO_2 was then added to bring the final partial pressure of CO_2 to 40 mm. (that of our alveolar air). The saturator was then placed in a water-bath accurately regulated to 38° and rotated for several minutes, after which a sample

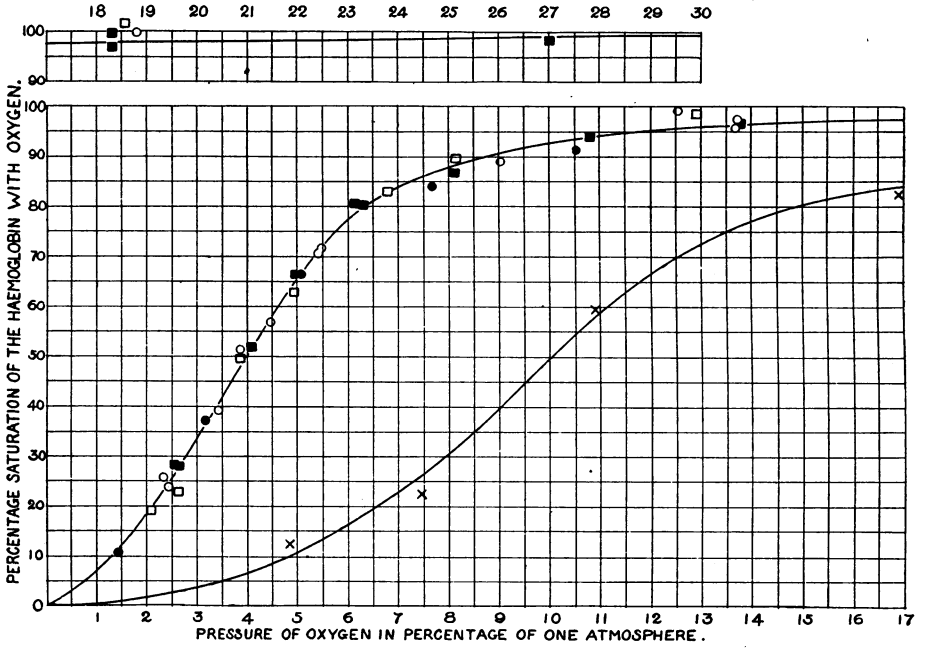

Fig. 3. Dissociation curves of oxyhæmoglobin in presence of 40 mm. pressure of CO_2 at 38°.

○ Blood of C. G. D., using ammonia in blood-gas apparatus.
● ,, ,, ,, Na_2CO_3 ,, ,,
□ ,, J. S. H., ,, ammonia ,, ,,
■ ,, ,, ,, Na_2CO_3 ,, ,,
× Mixed blood of six mice, using ammonia ,, ,,

of the gas in the saturator was taken for analysis, the excess of pressure (due to the rise of temperature) blown off, and the saturator rotated for a few minutes more. The blood was then collected in the neck of the saturator while it was still in the bath, the flask then removed, and a sample of the blood rapidly taken into a narrow graduated pipette,

avoiding all air-bubbles. Exactly 1 c.c. of this sample was delivered below the dilute ammonia or sodium carbonate solution in the bottle of the blood-gas apparatus. A trace of saponin was also added to make sure of laking. The bottle was then attached to the blood-gas apparatus, placed in the bath at room temperature, and a reading taken when it became perfectly steady. The blood was then agitated with the air of the bottle until the hæmoglobin was completely saturated, and another reading (twice repeated) taken. Ferricyanide was then added, and a third reading taken when all the oxygen had come off. In most of the experiments we placed dilute ammonia (0·4 % of strong ammonia) in the bottle. With this solution we found from blank experiments with fully saturated blood that a correction of 3·5 mm. of water was required in the second reading of the apparatus. This correction amounted to about 5 % of the total pressure given by the oxygen contained in the saturated blood, and as we were not quite convinced of the accuracy of so large a correction we finally adopted the plan of substituting 1 % sodium carbonate for the ammonia. This was the solution first used by Haldane in connection with the ferricyanide method of determining the oxygen of the blood, and it was with sodium carbonate that the original tests of the accuracy of the ferricyanide method were made[1]. Dilute ammonia was afterwards preferred on account of the difficulty in always laking the blood completely in presence of sodium carbonate. This difficulty is, however, overcome by using saponin; and the somewhat large correction necessitated by using ammonia is thus got rid of.

The partial pressures of oxygen and CO_2 in the saturator were calculated from the analyses and readings of the barometer, allowance being also made for the pressure of aqueous vapour in the saturator. In calculating the percentage saturation of the hæmoglobin we also made allowance for the gas which went into simple solution in the blood when the latter was saturated in the cold at about 15°, using the coefficients of absorption for oxygen, nitrogen and hydrogen in blood at 15° and 38° as given by Bohr. This correction amounted to from 3 to 5 % at different parts of the curve.

It will be seen that the blood of J. S. H. gives results practically identical with those given by the blood of C. G. D. The curve which summarises the results is also nearly identical with that already

[1] This *Journal*, xxv. p. 295. 1900. The correction when ammonia is used is necessitated by the diminished partial pressures of aqueous vapour and ammonia after the blood is mixed with the ammonia solution.

obtained by Barcroft[1] for the blood of C. G. D. There can, therefore, be hardly any doubt that Barcroft's curve is almost exactly correct. Our results were obtained in Oxford at various times since April 1910, but no certain variations in the curve could be detected, although it seems probable that slight variations occur. Results which we recently obtained during a stay of some weeks at an altitude of 14,100 feet will be described elsewhere.

In Fig. 3 we have also plotted a series of results obtained with the mixed blood of six mice. It will be seen that there is a very great difference between the mouse blood and human blood, and that in presence of an oxygen pressure of 100 mm. (13·1 % of an atmosphere) the mouse-blood was only about 74 % saturated. With regard to these determinations it should be remarked that the individual results are probably not so exact as in the case of human blood, as we only used 0·5 c.c. of blood for each determination. The blood was taken from the heart after the animals were killed by drowning. On account of asphyxial convulsions this blood must have contained an abnormal proportion of lactic acid; and this, as Barcroft and Orbeli have shown[2], would shift the curve very distinctly to the right.

In order to obtain some idea of the extent of this shifting we made the following experiment. A mouse was killed instantaneously by decapitation and the first blood which oozed out was collected and defibrinated. Of this blood ·05 c.c. was spread in a ring inside the saturator in the usual way, and another similar ring of fresh defibrinated blood from one of us was spread in a similar ring. The saturator was then filled with hydrogen, oxygen gradually added, 2 c.c. at a time, and the spectroscopic appearances observed after rotation of the saturator in the bath at 38°, and before the saturator had time to cool appreciably. The double band of oxyhæmoglobin became just faintly visible when the oxygen pressure in the saturator corresponded to 15 % saturation of the human blood, and this point was taken as an index. It was found that without CO_2 in the saturator about thrice as high an oxygen pressure was necessary in order to obtain the first appearance of the double band with mouse blood; but with 40 mm. of CO_2 pressure scarcely twice as much oxygen was needed for the mouse blood. This experiment gave the same result with similar blood from

[1] This *Journal*, XLII. p. 44. 1911. The agreement of our results with those of Barcroft confirm, also, the accuracy of the "differential" apparatus which he employed, using only 0·1 c.c. of blood for each determination.

[2] *Ibid.* XLI. p. 355. 1910.

another mouse, and we concluded that in presence of 40 mm. of CO_2 pressure mouse blood containing no abnormal proportion of lactic acid requires about 75% more oxygen pressure than human blood to produce a given percentage saturation of the hæmoglobin with oxygen. It would, therefore, take about 52·5 mm. of oxygen pressure to produce half-saturation of mouse blood in presence of 40 mm. of CO_2 pressure.

When tested with glazed litmus paper the mouse blood appeared to be distinctly less alkaline than human blood; and probably this is connected with the shifting of the dissociation curve to the right in mouse blood, and with the comparatively small effect of CO_2 on the curve. Further evidence for this view will be given later.

If it required about 52·5 mm. to produce half-saturation with oxygen in unaltered mouse blood in presence of CO_2, mouse blood will only be about 85% saturated in the arterial blood with the oxygen pressure at 100 mm., whereas human arterial blood is about 96% saturated. On the other hand the mouse blood must give off its oxygen to the tissues at a much higher pressure of oxygen than human blood, at any given percentage saturation of the venous blood. It seems probable that this difference is connected with the great increase in metabolism per unit of body weight in a small animal such as a mouse.

We have also determined the dissociation curve of CO-hæmoglobin in the absence of oxygen for the blood C. G. D., using the method already referred to above, except that the saturator was filled with pure hydrogen before the CO was introduced. Any excess of pressure in the saturator was allowed to blow off after a sample had been taken for determination of oxygen and CO_2. In calculating the results allowance was of course made for the pressure of aqueous vapour and the barometric pressure. As the hydrogen[1] (which was made from zinc and sulphuric acid and collected over water) contained a small proportion (about 0·2%) of oxygen, it was found that about 0·2% of oxygen was always present in the saturator. At the lower part of the curve this would increase and at the upper part diminish the saturation of the blood with CO, so that a small correction, calculated in the manner explained below, and greater when CO_2 was absent, had therefore to be made.

Fig. 4 shows the results obtained (1) in the absence of CO_2, and in the presence of (2) 19 mm., (3) 42 mm., and (4) 79 mm. pressure of CO_2. It will be seen that the dissociation curve in presence of 40 mm.

[1] Several samples of commercial hydrogen or nitrogen in cylinders were found by the blood test to be contaminated with CO, and were thus useless.

of CO_2 is similar in form to the curve for oxyhæmoglobin with 40 mm. of CO_2, allowance being made for the difference in the scale of abscissæ. The pressure required to produce half-saturation is 0·017 % of an atmosphere (0·13 mm. of Hg) for CO, and 4·0 % (30·4 mm.) for O_2. These pressures are in the ratio of 1 : 235, while at half-saturation in the curve for the blood of C.G.D. in Fig. 1 the ratio of CO to O_2 is 1 : 246.

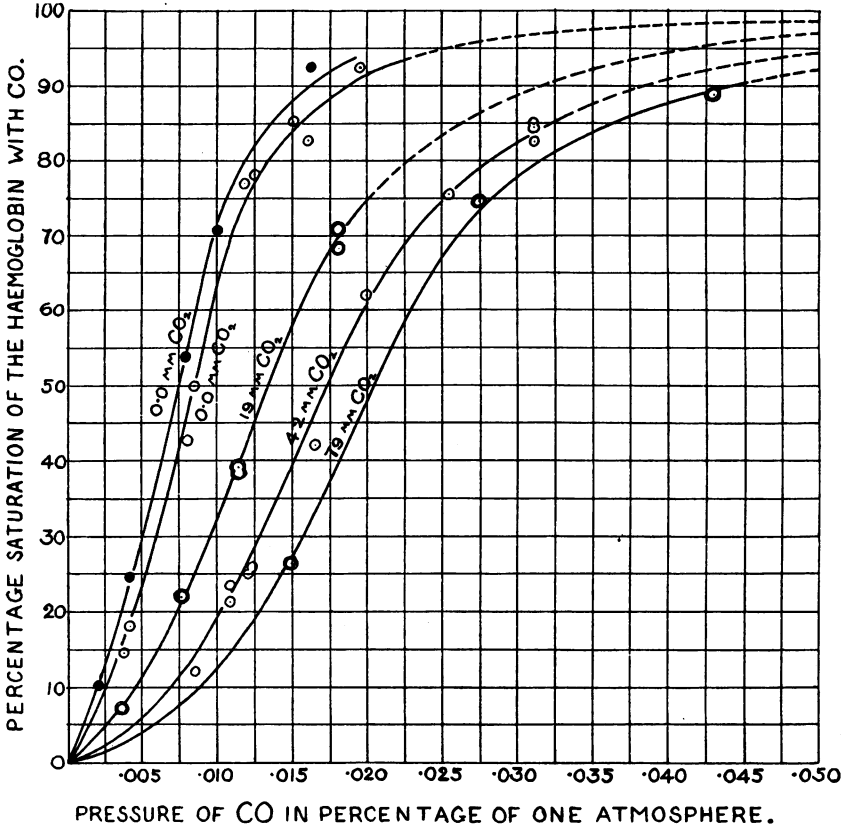

Fig. 4. Dissociation curves of CO-hæmoglobin in absence of oxygen, at 38° and with various pressures of CO_2.

○ Blood of C.G.D. ● Blood of J.S.H.

The ratios are nearly the same, as might be expected, and the small difference between them may be due to experimental errors.

On comparing the different curves in Fig. 4, it will be seen that they are to all appearances identical, if the scale on which the abscissæ of each are plotted is altered by a suitable constant. It thus appears that

the effect of varying proportions of CO_2, and presumably of acidity or alkalinity in general, is only to alter the scale on which the abscissæ are plotted; and since the dissociation curves shown in Fig. 1 are not affected by varying the pressure of CO_2 it follows that the effect of CO_2 etc. on the oxyhæmoglobin curve must be the same as on the curve for CO-hæmoglobin.

It will also be seen that given increases in the partial pressure of CO_2 produce less and less absolute and relative effect as the pressure of CO_2 rises. This result accords with the observations of Bohr, Hasselbalch, and Krogh, and of Barcroft and his associates, on the effects of CO_2 on the dissociation curve of oxyhæmoglobin. Our curves give the effect with greater precision, and for human blood.

In the same figure we have plotted the dissociation curve of CO-hæmoglobin in the absence of CO_2 for the blood of J. S. H. Although the curves for oxyhæmoglobin are the same, or nearly the same, for the blood of C. G. D. and J. S. H., as already shown, the percentage of CO required to produce half-saturation in presence of air is about 15 % less for the blood of J. S. H. than for that of C. G. D., as shown in Fig. 1. From these facts it might be expected that the dissociation curve of CO-hæmoglobin for the blood of J. S. H. would be about 15 % to the left of that of C. G. D. It will be seen that this is actually the case, so that the difference shown in Fig. 1 is due to the fact that the affinity for CO of the blood of J. S. H. is about a sixth greater than in the case of the blood of C. G. D.

In Fig. 5 are shown some results obtained for the dissociation of CO-hæmoglobin in mouse blood, the mice having been killed by drowning. It will be seen that nearly four times as much CO was required in order to produce half-saturation in the mouse blood as compared with human blood, while, as shown in Fig. 3, only about $2\frac{1}{2}$ times as much oxygen was needed to give half-saturation in similar mouse blood. The ratio between the pressures of CO and of oxygen required for half-saturation was thus about 1 : 148 for mouse blood; and this ratio corresponds with that between CO and O_2 at half-saturation in the upper curve for mouse blood in Fig. 2. Relatively speaking the dissociation curve for CO-hæmoglobin is shifted further to the right in mouse blood as compared with human blood than the curve for oxyhæmoglobin, and this explains the fact that in presence of air more CO is needed to produce a given saturation of mouse hæmoglobin with CO. The smallness of the influence of CO_2 on the curve for mouse blood is rather remarkable, but may be due to the fact that the

blood used was abnormally charged with lactic acid, so that the further increase in acidity due to the CO_2 had very little effect.

From the fact that neither the presence nor absence of a pressure of 40 mm. of CO_2, nor the addition to the blood of an equal volume of 1 % sodium carbonate solution, had any effect on the "mixed" dissociation curves in Fig. 1, we may also conclude that small differences

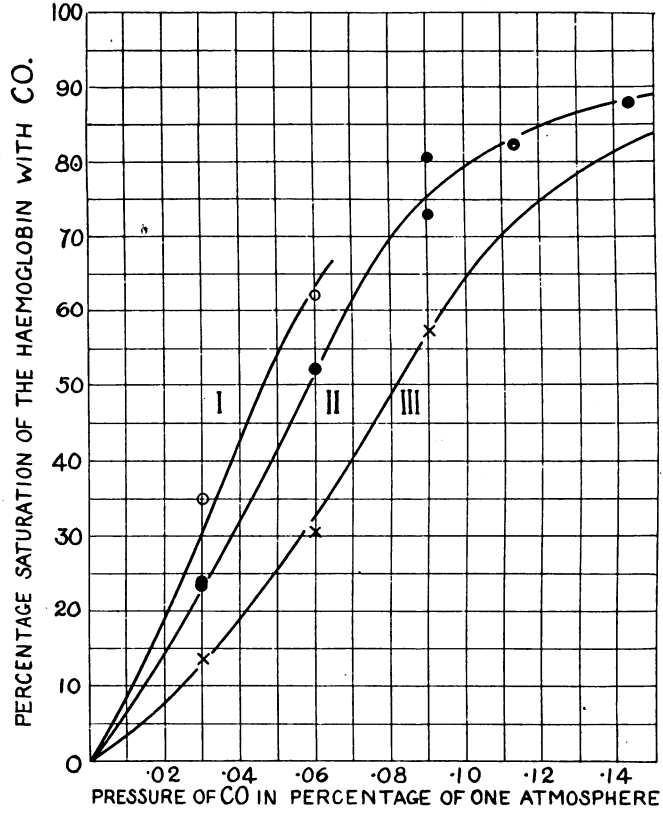

Fig. 5. Dissociation curves of CO-hæmoglobin in absence of oxygen, at 38°.

I. Blood of mouse *E*, no CO_2 present.
II. ,, ,, ,, *E*, 40 mm. pressure of CO_2 present.
III. ,, ,, ,, *F*, ,, ,, ,, ,,

in alkalinity or acidity of the blood affect the affinities for hæmoglobin of CO and O_2 equally.

We now come to the further analysis of the hump shown in the lower curves of Fig. 2. The upper curve was obtained with human blood in the presence of ·0945 % of CO, and it will be seen from the

dissociation curve of human blood in the absence of CO_2 (Fig. 4) that in the presence of as much as ·0945 % of CO the hæmoglobin will be completely saturated with CO in the entire absence of oxygen. There could therefore be no hump in the upper curve; and in the absence of O_2 and CO_2 it was actually found that 100 % saturation (within the limit of experimental error) was reached with less than half as much CO in the gas contained in the saturator.

In the case of the curves for mouse blood in Fig. 2 the conditions were very different; for in the absence of oxygen the hæmoglobin of this blood could only become about 65 % saturated in the presence of ·090 % CO, and 52 % saturated in the presence of ·063 %. To judge from the simple dissociation curves of mouse blood (Figs. 3 and 5) the mouse hæmoglobin would still be very incompletely saturated with CO and O_2 together until the pressure of oxygen rose to over 10 %; and the peculiar form of the hump at once suggests the theory that the deviation from the rectangular hyperbola depends upon the proportion of reduced hæmoglobin present, and that the relative proportions of CO-hæmoglobin to oxyhæmoglobin remain all the time dependent on the relative partial pressures of CO and O_2. The results for mouse blood were in accordance with this theory so far as the matter could be tested without exact knowledge of the separate dissociation curves for O_2 and CO of the samples of blood investigated; but in order to test the theory more thoroughly we have used the blood of C. G. D., of which the separate dissociation curves have already been given. The experiments were made in the presence of a pressure of 40 mm. of CO_2, as the data were required for the control of experiments on the arterial oxygen pressure in man; and for the same reason very low pressures of CO were used.

In Fig. 6 we have plotted the results of two series of experiments. The dotted rectangular hyperbola shows the curve which would have been obtained if the pressure of CO had been sufficient to saturate the hæmoglobin completely in the entire absence of oxygen. The lower curve (thick line) shows the line calculated on the theory that the ratio of oxyhæmoglobin to CO-hæmoglobin remains throughout (and without relation to the reduced hæmoglobin present) proportional to the partial pressures of oxygen and CO present, allowing for the fact that, as already shown in Fig. 1, the affinity of CO for hæmoglobin is 246 times as great as that of oxygen for hæmoglobin in the blood of C. G. D. The two upper curves (thin lines) show the calculated percentage saturation of the hæmoglobin with oxygen and CO together, the calculation being

based on the assumption that a given partial pressure of CO has 235 times as much effect as the same partial pressure of oxygen in saturating the hæmoglobin.

It will be seen at once that the experimental results coincide very closely with the calculated curve, so that the theory is certainly correct. We can thus calculate the deviation from the rectangular hyperbola provided that we know the simple dissociation curves for oxyhæmoglobin and CO-hæmoglobin of the blood used. In Fig. 7 four more

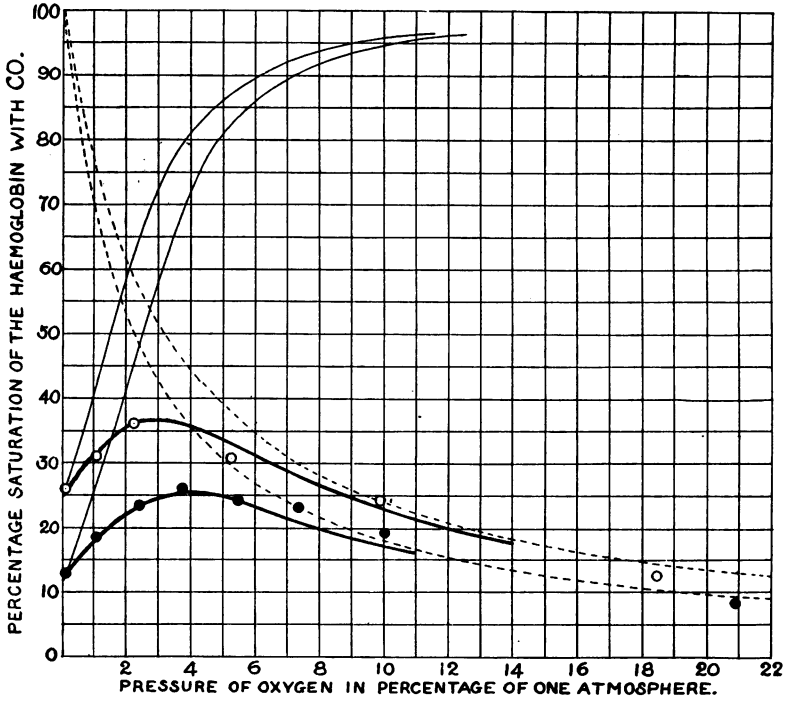

Fig. 6. Dissociation curves of CO-hæmoglobin at 38° and in presence of 40 mm. CO_2, with constant pressure of CO and varying pressures of oxygen. Blood of C. G. D.

○ CO = 0·0122 % atmosphere. ● CO = 0·00854 % atmosphere.

of these curves have been drawn in order to represent the conditions when the blood in the absence of O_2 is 5 %, 35 %, 50 % and 75 % saturated with CO.

It appears from the curves that in presence of low partial pressures of CO the hæmoglobin of blood may take up more than twice as much CO with a low partial pressure of oxygen as when oxygen is entirely absent. There can be no doubt that the converse is also true,

namely that in presence of a low partial pressure of oxygen the hæmoglobin will take up more than twice as much oxygen when a low partial pressure of CO is present as when no CO is present; and even when the blood is half-saturated with CO the intake of oxygen will be helped rather than hindered if the oxygen pressure is very low. The proportions of oxygen actually taken up can be read off from the differences between the corresponding upper and lower continuous curves in Figs. 6 and 7. This affords a very satisfactory explanation

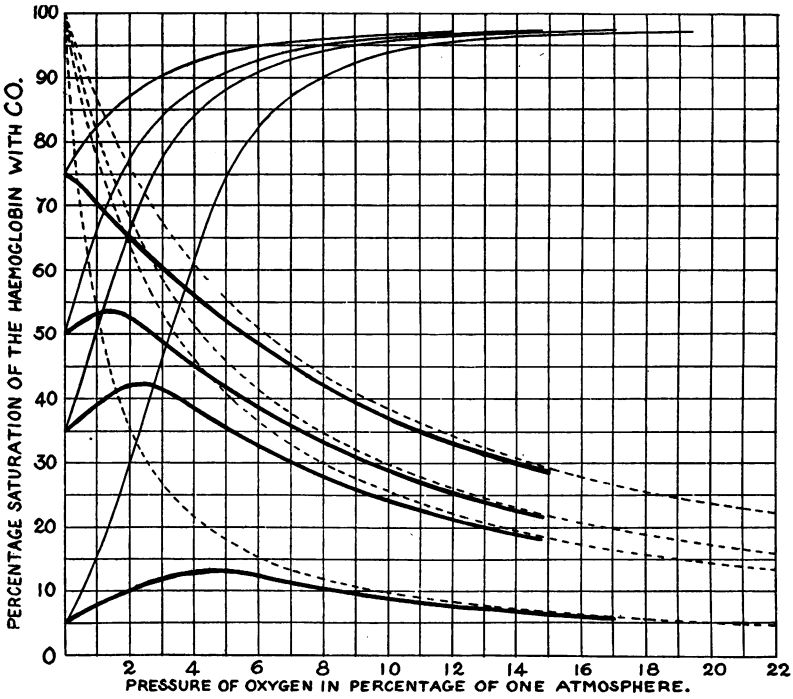

Fig. 7. Dissociation curves of CO-hæmoglobin in blood at 38° and in presence of 40 mm. CO_2, with constant pressure of CO and varying pressures of oxygen.

of the observation of Haldane and Lorrain Smith[1] that the administration of a certain amount of CO diminished, or at any rate did not increase, the symptoms of want of oxygen observed in mice on great diminution of the atmospheric pressure.

Haldane and Lorrain Smith[2], in their experiments on mice subjected to low partial pressures of oxygen, calculated the arterial oxygen pressure on the assumption that the partial pressure of the CO

[1] This *Journal*, XXII. p. 252. 1897. [2] *Ibid.* p. 244.

in the air breathed by the animals was sufficient to saturate their blood with CO in the complete absence of oxygen. The data given above show that this assumption was incorrect, as mouse blood differs greatly in this respect from ox blood or human blood, with which their test experiments outside the body were made. In the interpretation of their experiments they were also misled completely by reliance on the dissociation curve of oxyhæmoglobin as given by Hüfner in 1891. From this curve, and the arterial oxygen pressures as calculated from their experiments with carbon monoxide, it appeared as if the hæmoglobin in the animals' arterial blood must have been almost saturated with oxygen even at the point where asphyxia from want of oxygen was imminent; and the only valid method of explaining their cyanosed appearance etc. seemed to be that the arterial blood, although nearly saturated with oxygen in the lungs, was nevertheless charged with "reducing substances" which rapidly consumed the oxygen before more than a part of it could reach the tissues.

The data already given show that the pressure of oxygen required to half-saturate the hæmoglobin of mouse blood is fully twenty times greater than was shown in Hüfner's curve; and in the light of our present knowledge there is no difficulty in explaining the asphyxial symptoms as a result of low percentage saturation of the hæmoglobin in the arterial blood. On the other hand it seems almost inconceivable that the animals could have remained alive unless the arterial oxygen pressure had been raised above that of the alveolar air. For example, in the experiment quoted in full at the end of Haldane and Lorrain Smith's paper, the atmospheric pressure was reduced to 210 mm. before any carbon monoxide was added to the air, and the alveolar oxygen pressure of the mouse cannot have been more than 15 to 20 mm., at which oxygen pressure the blood would only have been 8 to 10 % saturated with oxygen. A mouse in which the oxygen carried by the arterial hæmoglobin is reduced as much as this by carbon monoxide poisoning is killed at once, although the conditions are otherwise much more favourable, since the arterial oxygen is at a far higher pressure. There seems, therefore, to be no doubt that the oxygen pressure in this and the other similar experiments must have been a good deal higher in the arterial blood than in the alveolar air, although the actual method of measuring the arterial oxygen pressure was fallacious.

The data and theoretical considerations embodied in Figs. 6 and 7 throw considerable new light on the symptoms of carbon monoxide

poisoning. The upper curves, representing the total saturation of the blood, also represent approximately (but not exactly) the forms of the dissociation curves of the oxygen left in combination with hæmoglobin when the latter is saturated with carbon monoxide to the percentage shown at the left end of each curve. It is clear from these curves that oxygen is given off from oxyhæmoglobin in the tisssues in a totally abnormal manner when the blood is highly saturated with carbon monoxide. Not only is the total quantity of oxygen given off diminished in proportion to the saturation with carbon monoxide, but the dissociation of the oxygen is altered in such a way that the oxygen comes off less readily, or at a lower pressure than in normal blood, and that most of it comes off at a very low oxygen pressure, instead of at a moderate oxygen pressure, as under normal conditions. The facts that in carbon monoxide poisoning the symptoms increase very gradually, and that a comatose condition intervenes between moderate symptoms and actual death, are thus rendered much more intelligible. The contrast between the helpless condition of a person whose blood is half-saturated with CO and the comparatively slight symptoms when the hæmoglobin is reduced to half its normal percentage in anæmia, is also elucidated.

It is evident from the results plotted in Figs. 1, 2, and 6, that if Haldane and Lorrain Smith's method be used for calculating the oxygen pressure in arterial blood from the percentage saturation of the blood with CO when the pressure of CO in the inspired air is known, we must base the calculation on observations outside the body as to the behaviour of the blood of the particular animal experimented on: also on the actual curves shown in Figs. 2 and 6, and not on the ideal rectangular hyperbolas shown in the same figures. This latter distinction becomes significant when the combined pressure of O_2 and CO becomes insufficient to saturate the hæmoglobin completely; and it should be noted that this point will be reached sooner in the presence of CO_2 than in its absence. The safest plan is to compare, if possible, the percentage saturation of the blood inside the body with the percentage saturation of the same blood outside the body in presence of a gas mixture having the same composition as the animals' alveolar air. Otherwise a proper allowance must be made in cases where the combined pressure of O_2 and CO is so low that the actual curve deviates from the ideal rectangular hyperbola. For human blood this allowance begins to become appreciable at a pressure of about 10 % of an atmosphere of oxygen when the blood is not more than 15–25 % saturated with CO. With mouse blood the allowance becomes appreciable at about 17 % oxygen pressure, or 12 %

if the saturation with CO is higher. It is evident, also, that at oxygen pressures of less than about half of these the method ceases to give reliable results under any circumstances, as the point is approaching where the percentage saturation of the blood with CO ceases to increase with diminution of the oxygen pressure, or where (in consequence of the "hump" in the curve) two results are possible, of which the lower or higher cannot be excluded with certainty. The application of these considerations to actual experiments will be referred to in the succeeding paper.

We must now endeavour to reach some explanation of the facts relating to the various dissociation curves discussed above.

It appears to us that in seeking an explanation we must start from the fact demonstrated by Barcroft and Roberts[1] that in the complete absence of salts the dissociation curve of oxyhæmoglobin (and doubtless also of CO-hæmoglobin) is a rectangular hyperbola corresponding to the simple reversible reaction,

$$Hb + O_2 \rightleftarrows HbO_2.$$

The actual dissociation curve given by the oxyhæmoglobin of blood must presumably be a rectangular hyperbola distorted in some manner which is dependent on the presence of salts. It is also to be noted that the curve for blood *cuts* the rectangular hyperbola for oxyhæmoglobin free from salts and CO_2, and does not merely diverge from it in a downwards direction, as we at first suspected might turn out to be the case. At above a certain percentage saturation the rectangular hyperbola is displaced upwards, and below this it is displaced downwards, and to a relative extent which increases so rapidly that the characteristic double bend is produced.

To explain the peculiar form of the curve Bohr[2] put forward the theory that oxyhæmoglobin does not merely dissociate into hæmoglobin and oxygen, but that the hæmoglobin also dissociates into its iron-containing portion (presumably hæmochromogen) and globin. He showed that a secondary reversible reaction of this type was capable of explaining the form of the curve. Apart from other difficulties, the spectroscopic evidence and the difficulty of understanding how hæmochromogen could exist as such in the presence of free oxygen seem to be definitely inconsistent with this theory.

It is known from the researches of Bohr, Barcroft, and their colleagues that CO_2 and other acids diminish the affinity of hæmoglobin

[1] This *Journal*, XXXIX. p. 143. 1909.

[2] *Nagel's Handbuch*, I. p. 73. 1905.

for oxygen, and if reduced hæmoglobin had the power of combining with alkali, and so neutralising it, we should have another conceivable explanation of the form of the dissociation curve for blood. Against this hypothesis, however, stands the fact—established by Bohr[1] that the presence or absence of oxygen in blood has no influence on its power of combining with carbon dioxide at any given partial pressure of the latter gas. We have also been unable to detect any difference in the reaction of serum from reduced, as compared with that from fully oxygenated, blood.

Another, and much more hopeful, theory has been proposed by Hill[2]. He considers that the form of the curve may be due to the existence, in the presence of salts, of a greater or less amount of aggregation among the molecules of hæmoglobin and oxyhæmoglobin, with the result that the reactions occurring may be represented by equations of the type

$$Hb + O_2 \rightleftarrows HbO_2$$
$$Hb_2 + 2O_2 \rightleftarrows Hb_2O_4$$
$$Hb_3 + 3O_2 \rightleftarrows Hb_3O_6.$$

He has also shown that, assuming the percentage aggregation to remain the same at all stages of dissociation, a general form of equation can be deduced, which, when proper constants are selected, will give the actual dissociation curves obtained by Barcroft with solutions of oxyhæmoglobin in the presence of salts. In his published paper Hill did not give an equation for the curve of oxyhæmoglobin in whole blood; but in a private letter he kindly communicated to us the required constants, and the equation fitted the experimental curve with great accuracy. Unfortunately, however, Hill's assumption in the particular form which he gave to it, is not consistent with the form (a rectangular hyperbola) of the dissociation curve of CO-hæmoglobin in presence of a constant partial pressure of oxygen; and there are also other improbabilities. On the other hand he has given solid reasons in favour of the idea that aggregation in some form does occur.

We think that all the facts can best be explained on the following assumptions: Aggregation of oxyhæmoglobin occurs, in the presence of salts, in accordance with the reactions:

$$HbO_2 + HbO_2 \rightleftarrows Hb_2O_4$$
$$HbO_2 + Hb_2O_4 \rightleftarrows Hb_3O_6$$
$$HbO_2 + Hb_3O_6 \rightleftarrows Hb_4O_8, \text{ etc.}$$

[1] *Nagel's Handbuch*, I. p. 106. 1905.

[2] This *Journal*, XL. p. iv. 1910.

and similarly for CO-hæmoglobin and reduced hæmoglobin. This aggregation occurs in accordance with the laws of mass action, and therefore increases or diminishes with increase or diminution of the concentration of oxyhæmoglobin, CO-hæmoglobin or reduced hæmoglobin; but the tendency to aggregation is greater in the case of the unsaturated molecules of reduced hæmoglobin than in that of the saturated molecules of oxy- or CO-hæmoglobin. The latter molecules also aggregate together to form Hb_2O_2CO, $Hb_3(O_2)_2CO$, etc., the molecules of HbO_2 having just as much tendency to aggregate with those of HbCO as with those of HbO_2. The aggregated molecules do not give up or take up O_2 or CO without first splitting up into simple molecules.

It will be seen at once that this hypothesis affords a general explanation of the facts. At the upper part of the dissociation curve of oxyhæmoglobin in blood the primary rectangular hyperbola of salt-free oxyhæmoglobin is distorted in an upwards direction on account of the large extent of aggregation of the relatively concentrated solution of oxyhæmoglobin. In consequence of this aggregation the active mass of oxyhæmoglobin (*i.e.* the concentration of unaggregated oxyhæmoglobin) is much less than it would otherwise be, and a greater total relative amount of oxyhæmoglobin in the aggregated and unaggregated state will be present than if none of the oxyhæmoglobin were aggregated. Similarly, at the lower part of the blood curve the percentage aggregation of reduced hæmoglobin will be greatly increased, while the percentage aggregation of oxyhæmoglobin will be correspondingly diminished, so that the primary rectangular hyperbola will be distorted downwards. By assuming sufficient proportions of aggregation among the molecules of oxyhæmoglobin and reduced hæmoglobin respectively the rectangular hyperbola will be converted into a double-bended curve; and by assuming that the percentage of aggregation is greater among the molecules of reduced hæmoglobin than among those of oxyhæmoglobin we can account for the fact that the blood curve cuts the primary rectangular hyperbola high up.

A similar explanation will, of course, apply to the simple dissociation curve of CO-hæmoglobin in blood. The case of the dissociation curve of CO-hæmoglobin in presence of a constant pressure of oxygen (sufficient to saturate the hæmoglobin with $O_2 + CO$) or the converse case, is met by the assumption that the saturated molecules of oxyhæmoglobin and CO-hæmoglobin molecules aggregate with one another just as readily as do either oxyhæmoglobin molcules with one another or

CO-hæmoglobin molecules with one another. In consequence of this the percentage aggregation of oxyhæmoglobin and CO-hæmoglobin remains the same at all parts of the dissociation curve, which is therefore a rectangular hyperbola, as is actually the case. In the case, finally, where the pressure of oxygen and CO together is insufficient to saturate the hæmoglobin, the dissociation curve, so far as reduced hæmoglobin is concerned, will be the same as when oxyhæmoglobin alone, or CO-hæmoglobin alone, is present at a pressure equivalent in saturating power to that of the O_2 and CO together; and the oxygen and CO will divide their combined share of the hæmoglobin in just the same proportions as if they together combined with the whole of the available hæmoglobin. This, as we have seen in connection with the "hump" curves is what actually happens.

If this theory is correct it follows that the proportion of aggregation must diminish with dilution of blood, or of any hæmoglobin solution in presence of salts; and consequently the simple dissociation curves of oxyhæmoglobin or CO-hæmoglobin will approximate more to their primary rectangular hyperbolas. We have not determined dissociation curves of oxyhæmoglobin in diluted blood (for which a special colorimetric method would be needed) but we have found that undiluted blood in presence of so low a pressure of oxygen that it shows no double spectrosopic band at the body temperature[1], becomes less blue, and shows a very strong double band when it is diluted largely with water, and saturated at the same temperature and oxygen pressure. We obtained this result with human blood in presence of a pressure of 0·7 % of an atmosphere of oxygen, and with mouse blood in presence 2·5 % of oxygen, no CO_2 being present in either case. We also found that a strong double band was still present when strong saline solution was added so that 1 % of NaCl was present in the blood solution. These results correspond to the theory. In the case of the dissociation curve of CO-hæmoglobin we endeavoured to obtain quantitative results, but the data were not very satisfactory, owing to the difficulty in avoiding the presence of traces of oxygen in the saturator, and preventing the decomposition of the blood solution during the period of time required for saturation. The approximate results obtained, however, showed that with about ·003 % of CO in the saturator a 1 % blood solution (blood of J.S.H.) became about half-saturated with CO, wheras undiluted blood became only about 12 % saturated. On doubling the

[1] When hæmoglobin is less than about 15 % saturated with O_2 the double band becomes invisible.

percentage of CO the blood solution became about two-thirds saturated, so that the curve for the diluted blood was apparently a rectangular hyperbola, as required by the theory.

The curves in Fig. 4 (showing the effects of CO_2 on the dissociation of CO-hæmoglobin) indicate that differences in reaction do not appreciably affect the amount of aggregation in the hæmoglobin, but do affect the affinity of hæmoglobin for CO or O_2. We should therefore expect (on the theory just stated), that differences in reaction would affect the dissociation curve in dilute solution just as much as in undiluted blood. To test this point we made the following experiment:—About ·05 c.c. of undiluted blood of J. S. H. was spread in a ring inside the saturator, which was then filled with hydrogen, 2 c.c. of air added, and the saturator rotated at 38° for a few minutes. On examination no trace of a double band could be seen in the blood. 10 c.c. of distilled water were then introduced, so as to dilute the blood to $\frac{1}{200}$th, and the rotation at 38° repeated. On examining the blood solution (in a suitably thick layer in the neck of the saturator) the double band of oxyhæmoglobin was now just faintly visible, corresponding to something like 20 % saturation. 1 c.c. of a 4 % solution of ordinary strong ammonia was now introduced, so that nearly 0·4 % of the strong ammonia was present in the blood solution. The rotation and subsequent examination were again repeated, with the result that the double band was now present strongly, and the hæmoglobin appeared to be about half-saturated with oxygen. The oxygen percentage in the saturator was then determined, and found to be 0·29 %. This experiment proves clearly that the alkali increased greatly the affinity of hæmoglobin for oxygen in the very dilute solution of blood.

The same experiment was repeated with mouse blood, and with the same result. The oxygen percentage at the end was 0·25. It was somewhat of a surprise to us to find that the double band was about equally visible in the dilute mouse blood after simple dilution with water. This result seems to indicate that the shifting of the dissociation curve to the right in undiluted mouse blood as compared with human blood is due to diminished alkalinity of the mouse blood, or at any rate not to differences between the molecules of mouse hæmoglobin and those of human hæmoglobin.

We must finally consider the very interesting question as to the cause of the differences in the relative affinities of CO and O_2 for hæmoglobin in the blood of different individuals, whether of the same or

of different species. We have seen that the relative affinities are distinctly influenced by temperature, rise of temperature diminishing the affinity of hæmoglobin for CO more than an equal rise diminishes the affinity for O_2. Light has a far more marked differential effect, as was pointed out by Haldane and Lorrain Smith, whose observations have been recently extended by Hasselbalch. It might, perhaps, have been expected that differences in reaction and in the salts present in the corpuscles of different species or individuals would have a similar effect, and would suffice to explain the differences in relative affinities. We have seen, however, that the differences remain, in spite of alterations in the reaction, and in spite of dilution to 1% with water. It seems, therefore, impossible to come to any other conclusion than that the differences in question are due to differences in the hæmoglobin molecules in different individuals. The difference can hardly lie in the hæmochromogen part of the molecule as there is good evidence that this is identical in all varieties of hæmoglobin. The ratio of oxygen capacity to colour is constant, as was shown by Haldane and Lorrain Smith, whose results have been confirmed by others; and Peters[1] has recently shown by an improved method of iron determination that the ratio of iron to oxygen capacity is also constant. In any case the difference is hardly likely to lie in the relatively simple hæmochromogen part. It is much more probable that the very complex globin part is variable and that the average constitution of this part differs in different individuals.

This conclusion as to the existence of differences in the hæmoglobins of different individuals, even of the same species, is of much general interest: for probably there are similar individual differences in other proteins which, when tested by the methods hitherto available, appear to be identical in different individuals. Hence, if we look at the body simply from the abstract chemical standpoint, we must conclude that not only is the arrangement of molecules different in different individuals, but the corresponding molecules themselves may differ also.

In calculating the amount of aggregation in the hæmoglobin of undiluted human blood, and the precise influence of this aggregation on the dissociation curve, we are somewhat at a loss, since it is not yet known what curve human hæmoglobin solution would give in the absence of salts, and what disturbing influence would be produced by the fixed alkalies of the blood in one direction and by carbonic acid in the other direction. We have provisionally assumed, however, that but

[1] This *Journal*, XLIV. p. 131. 1912.

for the influence of aggregation the oxyhæmoglobin of human blood in the presence of 40 mm. pressure of CO_2 would give as its dissociation curve a rectangular hyperbola with 50 % saturation corresponding to a partial pressure of 1·6 % of an atmosphere (12·2 mm. of oxygen).

Taking x as the pressure of O_2 in hundredths of an atmosphere, and y as the fraction of the hæmoglobin oxidized (the whole being taken as unity), the equation of this curve is $x = \frac{1{\cdot}6y}{1-y}$.

To allow for the aggregation, let y_1 be the amount of HbO_2 present in single molecules, y_2 the amount of $(HbO_2)_2$, y_n the amount of $(HbO_2)_n$. Similarly let z_1 be the amount of Hb, z_2 the amount of Hb_2, and so on. Then, since $2HbO \rightleftarrows (HbO_2)_2$ is a simple reversible reaction, y_2 must vary as y_1^2, and y_n as y_1^n, whether the reaction be $HbO_2 + (HbO_2)_{n-1} \rightleftarrows (HbO_2)_n$, $nHbO_2 \rightleftarrows (HbO_2)_n$, or otherwise. Similarly z_2 must vary as z_1^2 and so on.

Hence
$$y = y_1 + y_2 + y_3 + \ldots$$
$$= y_1 + a_1 y_1^2 + a_2 y_1^3 + \ldots.$$

Similarly $1 - y = z_1 + b_1 z_1^2 + b_2 z_1^3 + \ldots$, a_1, a_2, b_1, b_2, etc. being quantities which depend on the velocities of the various aggregations, and are constants for any given concentration of salts. For human blood we may assume that $a_1 = 2$, $a_2 = 2^2$, $a_n = 2^n$, and $b_1 = 8$, $b_2 = 8^2$, $b_n = 8^n$, *i.e.* that the masses of HbO_2, $(HbO_2)_2$, $(HbO_2)_3$ etc. are always in a geometrical progression, and similarly for those of Hb_1, Hb_2, Hb_3, etc. There is no *a priori* reason for this assumption, but it leads to a simple formula which agrees fairly accurately with our experimental results.

Since $y = y_1 + 2y_1^2 + 2^2 y_1^3 + \ldots.$

$$= \frac{y_1}{1-2y_1} \quad \therefore \; y_1 = \frac{y}{1+2y}, \text{ and similarly } z_1 = \frac{1-y}{1+8(1-y)}.$$

Now since it is only the single Hb and HbO_2 molecules that combine with or give off oxygen, the equation to the dissociation curve, allowing for aggregation, is

$$x = \frac{1{\cdot}6y_1}{z_1}$$
$$= \frac{1{\cdot}6y\,(9-8y)}{(1-y)\,(1+2y)}.$$

This formula gives the results shown in the accompanying table. The first column gives pressures of O_2 in % of an atmosphere, *i.e.* x: the second gives the calculated percentages, *i.e.* $y \times 100$: the last, the

percentages read off from the curve of Fig. 3. Considering the range of experimental error, the agreement is pretty close.

Pressure of O_2 in % of an atmosphere	% saturation calculated	% saturation read off on curve
0	0	0
·66	5	4
1·21	10	9
2·11	20	19·5
2·83	30	30·5
3·44	40	40·5
4·00	50	50
4·58	60	59
5·29	70	69·5
6·40	80	80·5
7·39	85	85·5
9·25	90	91
14·68	95	96·5
25·4	97·5	98·5

It seems probable that all absolute dissociation curves both of HbO_2 and HbCO can be represented by the equation $x = \frac{Ky[1-b(1-y)]}{(1-y)(1+ay)}$, where K, a, and b are suitable constants. The values of a and b increase with increase of salt concentration, but are not appreciably affected by acidity or alkalinity. The values $a = 2$, $b = 8$, mean that in human blood saturated with O_2, $\frac{2}{3}$ of the oxyhæmoglobin is aggregated and $\frac{1}{3}$ free, and in completely reduced blood, $\frac{8}{9}$ of the reduced hæmoglobin is aggregated, and $\frac{1}{9}$ free.

The value of K is increased by acidity, and Barcroft's results with neutral salts[1] suggest that they also may affect it. Thus the values of K for HbCO in the blood of C.G.D. are, for 0 mm. pressure of CO_2, ·0034, for 19 mm., ·0054, for 42 mm., ·0069, and for 79 mm., ·0082. These results are in accord with those of Mathison[2] and Oinuma[3], who compared the rates of the reactions $HbO_2 \longrightarrow Hb + O_2$ and $Hb + O_2 \longrightarrow HbO^2$, at different pressures of CO_2. K is the ratio of the velocities of these reactions, and its increase by heat and acids is due both to the acceleration of the former reaction, and the retardations of the latter. Judging from the data for CO, the value of K for oxidation of hæmoglobin in human blood in absence of CO_2 is about ·8; while for a dialysed solution of dog's hæmoglobin in water, Barcroft's results give $K = 1·05$.

[1] Barcroft and Camis. This *Journal*, XXXIX. p. 123. 1909.
[2] Mathison. *Ibid.* XLIII. p. 347. 1911. [3] Oinuma. *Ibid.* p. 364. 1911.

For the same concentration of salts and acids, a and b (the constants which determine the rates of aggregation) are the same for HbCO as for HbO_2, but K is smaller for HbCO in a ratio which is constant for the hæmoglobin of any individual. For the blood of C.G.D. the ratio is about $\frac{1}{224}$, for that of J.S.H. $\frac{1}{290}$, for the mouse of Fig. 5, $\frac{1}{148}$.

CONCLUSIONS.

1. When a solution of hæmoglobin, whether enclosed in blood corpuscles or free, is saturated in presence of a gas mixture containing oxygen and carbon monoxide, the ratio of oxyhæmoglobin to CO-hæmoglobin is always proportional to the relative partial pressures of oxygen and carbon monoxide, and is not altered by the presence of CO_2, or slight changes in reaction, or of reduced hæmoglobin, or by dilution, but is appreciably altered by temperature, as well as by light, and varies distinctly in the hæmoglobin of different individuals and species.

2. The oxyhæmoglobin dissociation curves for the blood of two persons is given, and the results agree closely with Barcroft's. Curves are also given for mouse blood, which differ greatly from those for human blood.

3. Dissociation curves of CO-hæmoglobin in presence of varying partial pressures of CO_2 are also given for human and mouse blood, and it is shown that the results vary, not only for different species, but also for different individuals. The curve for CO-hæmoglobin has the same form as that for oxyhæmoglobin.

4. When blood is saturated in presence of such low partial pressures of oxygen and CO that reduced hæmoglobin is present, the proportions of oxyhæmoglobin, CO-hæmoglobin, and reduced hæmoglobin can be calculated if the separate dissociation curves of oxyhæmoglobin and CO-hæmoglobin are known; and in consequence of the form of these curves it follows that the presence of a small proportion of oxygen may greatly increase the formation of CO-hæmoglobin, and *vice versa*. This paradoxical effect explains the favourable physiological effect sometimes produced by carbon monoxide in conditions of great anoxhæmia. The dissociation curve of the oxyhæmoglobin remaining in the blood when it is partially saturated with carbon monoxide can also be deduced; and the form of this curve explains the peculiarities of the symptoms of carbon monoxide poisoning as compared with those of anoxhæmia from other causes.

5. A theory, based on A. V. Hill's "aggregation" hypothesis, and on the work of Barcroft and his associates, as well as on our own observations, is put forward to account for the peculiar form of the dissociation curve of oxyhæmoglobin and CO-hæmoglobin in blood or in salt-solutions, and the effects of CO_2 etc. on these curves. An equation for the curve, based on this theory, is also given, and is shown to correspond closely with the experimental data for human blood.

6. The differences in the relative affinities for oxygen and carbon monoxide in different samples of hæmoglobin indicate that the average constitution of the globin part of the hæmoglobin molecule varies, not only in different species, but also in different individuals of the same species.