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THE CARBON MONOXIDE DISSOCIATION CURVE OF HUMAN BLOOD

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The complete dissociation curve of carboxyhaemoglobin has not been studied since Douglas, Haldane & Haldane published their classic paper on the subject in 1912. Their results comprised four COHb dissociation curves on the blood of Douglas at CO_2 pressures of 0, 19, 42 and 79 mm Hg, respectively, and a single curve on Haldane's blood in the absence of CO_2. The CO content of the blood was estimated by a colorimetric method; the concentration of CO in the tonometer at equilibrium was not measured directly but was calculated from the amount introduced volumetrically before equilibration, corrected for the amount absorbed by the blood. In view of the small number of experiments performed and the limitations of the methods then available, it seemed desirable to repeat the investigation of Douglas *et al.* (1912), using the more accurate and convenient methods available at the present time. COHb dissociation curves have therefore been determined in the absence of oxygen, at three different CO_2 pressures, for the blood of three normal subjects. These curves have been compared with the corresponding O_2Hb curves.

METHODS

For each experiment 20–25 ml. of venous blood was freshly drawn into a syringe, the dead space of which was filled with heparin solution.

Deoxygenation of the blood. Before determining the carboxyhaemoglobin dissociation curves, the blood was deoxygenated as completely as possible, to exclude the effect of any residual oxyhaemoglobin on the uptake of carbon monoxide. 17 ml. of the blood was introduced into a 410 ml. tonometer which was evacuated to a pressure of 50 mm Hg. Further lowering of the pressure was found to cause boiling of the blood and undue haemoconcentration. Nitrogen was then admitted to the tonometer to restore the pressure to atmospheric. This was followed by a second evacuation and restoration of the pressure with nitrogen, after which the tonometer was rotated for 5 min in a water-bath at 37° C. The whole cycle was repeated four times making a total of eight evacuations and four 5 min periods of equilibration with nitrogen at 37° C. The

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residual oxygen content was then 0·1–0·3 ml./100 ml., which was comparable with that of residual carbon monoxide when the blood was similarly treated before determining the oxyhaemoglobin dissociation curves.

Gas mixtures and equilibration of the blood samples. 1·5 ml. of blood was used for the determination of each point on the dissociation curves. The appropriate gas mixtures of CO and N_2, or O_2 and N_2, were made up manometrically in 410 ml. tonometers from cylinders of these gases, sufficient CO_2 being added to bring the pCO_2 to 15, 40 or 70 mm Hg, as required. The use of a cylinder of 0·5% CO in N_2 instead of pure CO permitted more accurate measurement of the very small quantities of CO required. The N_2 used for the COHb experiments was of a high degree of purity, containing less than 1 part in 10,000 of O_2. For the determination of the O_2 and CO capacities the tonometers were made up to contain 21–23% O_2 in N_2, or 0·5% CO in N_2, plus the pCO_2 appropriate to the curve being determined. 1·5 ml. of the deoxygenated blood was then added to each tonometer which was rotated at 37° C in the water-bath. The inside of the bath was painted black and the top covered with a board to exclude the light during equilibration with CO.

Equilibration of the blood with O_2 was completed in 20–30 min, but 2–4 hr were required for equilibration with CO, the longer period being particularly necessary at the higher saturations. Since the CO capacity measured after equilibration for 90 min at 37° C was identical with that after equilibration for 4 hr, we felt justified in assuming that the absence of O_2 in the tonometer obviated any conversion of haemoglobin to methaemoglobin. After equilibration each tonometer was placed upright in the water-bath to allow the blood to drain. It was then removed from the bath and the blood taken without delay into a syringe, which was rotated in a mixture of ice and water while awaiting analysis. A gas sample was also taken into a Brodie bottle for CO_2 and O_2 analysis with the Scholander micro-gas analyser (Scholander, 1944). If a COHb curve was being determined the remaining gas was immediately analysed for CO. The tonometer was not allowed to cool before this analysis since the affinity of blood for CO is increased by a fall in temperature. Even though only 0·2–0·3 ml. of blood remained in the tonometer the CO percentage in the tonometer was so low that it was found to be appreciably reduced by the further uptake of CO if cooling was allowed to occur.

CO analysis. The tonometer gases were displaced by mercury through tubes containing soda asbestos and magnesium perchlorate, which removed CO_2 and water vapour, respectively. The observed CO percentages were subsequently corrected for the changes in volume resulting from the absorption of CO_2 and water vapour. The gases were then passed through an infra-red CO analyser (Infra-red Development Company, Type S.C.L.). The instrument was calibrated before use with a series of five standard CO mixtures and was also checked before and after the analysis of each tonometer. With these precautions the accuracy was $\pm 0{\cdot}0003$% of CO.

Blood analysis. The blood was analysed for O_2 and CO by the Roughton–Scholander syringe methods (Roughton & Scholander, 1943; Scholander & Roughton, 1943). The analysis was generally carried out within 10–15 min of withdrawing the blood from the tonometer. Several small modifications were introduced which increased the accuracy of the analysis to $\pm 0{\cdot}15$ ml./100 ml. These included (i) preliminary deoxygenation of the reagents by evacuation and subsequent equilibration with nitrogen, which reduced the blank for the O_2 determination from 0·9–1·2 ml./100 ml. to 0·1–0·2 ml./100 ml. and (ii) warming the sampling pipette before taking up the blood, thus ensuring more uniform and complete drainage. The same syringe and sampling pipette were used throughout the whole series of experiments, and the quantities of reagents used for each analysis were kept as constant as possible. The over-all improvement was such that duplicate determinations of CO or O_2 capacity, using two blood samples equilibrated in separate tonometers, were nearly always within 0·2 ml./100 ml. of one another.

The pH of each equilibrated sample was measured at 37° C using a capillary electrode system with a capacity of 0·1 ml. (Joels & MacNaughton, 1957) in conjunction with a Cambridge pH meter.

The haematocrit was measured by spinning the blood in Wintrobe tubes for 30 min at 4000 rev/min.

The specific gravity of the whole blood was estimated by the copper sulphate drop method (Phillips, Van Slyke, Hamilton, Dole, Emerson & Archibald, 1950).

The degree of haemolysis was also measured in a few experiments by spectrophotometric estimation of the haemoglobin content of the plasma. These last three measurements gave an indication of the degree of drying and red cell damage due to the repeated evacuation and prolonged equilibration. In general the haematocrit value rose by 1–2% and the specific gravity of the blood increased by 0·002 (e.g. from 1·060 to 1·062). The degree of haemolysis never represented more than 5% of the red corpuscles and was often much less. These changes were not excessive in view of the procedures to which the blood was subjected.

Correction of the observed results

Oxyhaemoglobin dissociation curves

The O_2Hb dissociation curves have been corrected (i) for dissolved O_2, (ii) for the presence of residual COHb, (iii) for haematocrit variations, and (iv) to bring the curves for all three subjects at each pCO_2 to the same plasma pH.

Dissolved O_2. This was calculated from the pO_2 of the gas in the tonometer and the solubility coefficient of O_2 in blood, given by Sendroy, Dillon & Van Slyke (1934) as 0·0031 ml./100 ml./mm pO_2 at 37° C. The result was subtracted from the O_2 content of the blood sample.

Residual COHb. The equilibrated blood samples of J.A., a moderately heavy smoker, were 2–4% saturated with COHb, and even though G.P. and N.J. were non-smokers their equilibrated samples also were between 0·5 and 1·5% saturated with COHb. The effect of this COHb on the O_2Hb dissociation curve was corrected for as described by Roughton & Darling (1944). The method is based on the following assumptions formulated by Douglas *et al.* (1912):

(*a*) that when blood is equilibrated with a mixture of O_2 and CO at pressures sufficient to cause all the haemoglobin to combine with O_2 and CO, the partition of the haemoglobin between COHb and O_2Hb is given by the equation $\frac{(COHb)}{(O_2Hb)} = \frac{M pCO}{pO_2}$, where M represents the relative affinities of haemoglobin for CO and for O_2;

(*b*) that the haemoglobin combined with gas is partitioned between COHb and O_2Hb according to the above equation even when reduced haemoglobin is present in appreciable quantities; and

(*c*) that the amount of reduced haemoglobin present after equilibration with a mixture of O_2 at a partial pressure pO_2 and CO at a partial pressure pCO, is the same as it would be in the absence of CO if the partial pressure of O_2 were $pO_2 + M pCO$.

The calculation can best be illustrated by working through a typical example. Suppose analysis of a blood sample shows $(O_2Hb) = 41·0\%$, $(COHb) = 1·3\%$ and (Reduced Hb) = 57·7%, and the corresponding gas analysis gives a pO_2 of 22·8 mm Hg. We wish to find x, the partial pressure of O_2, which would be in equilibrium with the blood when the (Reduced Hb) = 57·7% and the remainder of the haemoglobin is all present as O_2Hb.

From assumption (*c*),
$$x = pO_2 + M pCO = pO_2 \left(1 + \frac{M pCO}{pO_2}\right);$$

but from (*a*)
$$\frac{M pCO}{pO_2} = \frac{(COHb)}{(O_2Hb)} = \frac{1·3}{41·0};$$

therefore
$$x = pO_2 \left(1 + \frac{1·3}{41·0}\right) = 22·8 \left(1 + \frac{1·3}{41·0}\right) = 25·1 \text{ mm Hg.}$$

Thus the corrected values for this point would be
$$(O_2Hb) = 42·3\%, \quad pO_2 = 25·1 \text{ mm Hg.}$$

Haemocrit variations. Since the error in reading the haematocrit (±0·5%) is relatively greater than that of the blood gas analysis (±0·1 ml./100 ml.), corrections were only applied when the haemocrit differed by more than 1% from the mean haematocrit for that particular experiment. The true percentage saturation was obtained from the observed percentage saturation $\times \frac{\text{mean haematocrit}}{\text{observed haematocrit}}$.

pH correction. The pH values of the individual samples were rarely more than 0·03 pH unit from the mean for any particular experiment, and the mean pH values for the three subjects were

very similar at each pCO_2. However, the mean pH value for the COHb curves was always lower than that for the corresponding O_2Hb curves, owing to the longer period of equilibration with greater opportunity for glycolysis and lactic acid formation. All pH values were therefore corrected to those of the corresponding COHb curves, the mean of the three COHb curves being taken as the standard pH at that pCO_2. Thus all the 15 mm Hg pCO_2 curves, both O_2Hb and COHb, were corrected to pH 7·50, all the 40 mm pCO_2 curves to pH 7·25, and all the 70 mm pCO_2 curves to pH 7·15. The correction applied to the O_2Hb curves was the empirical relation of Dill, Graybiel, Hurtado & Tacquini (1940); $\Delta \log pO_2 = -0{\cdot}48\,\Delta$ pH.

Carboxyhaemoglobin dissociation curves

These have been corrected for (i) residual O_2Hb, (ii) haematocrit variations and (iii) pH. No correction was required for dissolved CO since the pCO in the tonometer was far too low for any appreciable quantity of CO to pass into solution.

Residual O_2Hb. The (O_2Hb) found on analysis of the blood samples was not thought to be a reliable index of the (O_2Hb) in the tonometer at equilibrium, since a small amount of oxygen might have been taken up by the blood while in the Roughton–Scholander pipette. Any tendency for the blood to take up oxygen at this stage of the analysis would be greatly enhanced by the increased affinity for oxygen in the presence of COHb. Instead, the correction was based on the small partial pressure of oxygen, pO_2, (0·5–2·0 mm), unavoidably present as a contaminant in the tonometer, since this pO_2 would have been in equilibrium with the blood. (O_2Hb) was calculated by the equation of Douglas *et al.* (1912), $(O_2Hb) = pO_2\,(COHb)/MpCO$, taking the value of 220 for M which preliminary examination of our data showed to be a reasonable approximation. (It will be seen later that the values for M based on the fully corrected data range from 223 to 267. Use of the value 220 produces a maximum error of 0·001 mm Hg in the corrected pCO; an error which is quite without significance.) Having found (O_2Hb), the sum of the concentrations (COHb) + (O_2Hb) was plotted against $(pCO + pO_2/M)$ in a similar manner to the oxyhaemoglobin dissociation curves.

Haematocrit variations were compensated for as described for the O_2Hb curves.

pH values for the COHb curves have been corrected to the standard pH at the appropriate pCO_2 using the relationship $\Delta \log (pCO \times 220) = -0{\cdot}48\Delta$ pH. This was derived from Dill's equation $\Delta \log pO_2 = -0{\cdot}48\Delta$ pH by substituting $\Delta \log (MpCO)$ for $\Delta\, pO_2$ and taking our approximate value of 220 for M. This substitution is justified by the finding of Parsons (1917) and Hastings, Sendroy, Murray & Heidelberger (1924) that combination with CO has exactly the same effect on the reaction of haemoglobin as has combination with O_2. An example will serve to illustrate the method of calculating this correction. The observed values for one point were 46·6% COHb at pCO = 0·123 mm Hg and pH of 7·18. For correction to the required standard pH of 7·25, Δ pH = +0·07. Therefore

$$\Delta \log (pCO \times 220) = -0{\cdot}48\Delta\ pH = -0{\cdot}48(+0{\cdot}07) = -0{\cdot}0336.$$

Now

$$\text{corrected log } (pCO \times 220) = \text{original log } (pCO \times 220) + \Delta \log (pCO \times 220)$$
$$= \log (0{\cdot}123 \times 220) + (-0{\cdot}0336) = 1{\cdot}4393 - 0{\cdot}0336 = 1{\cdot}4057.$$

Thus corrected pCO × 220 = antilog 1·4057 = 25·5; whence the corrected pCO = 0·116 mm Hg.

RESULTS

Oxyhaemoglobin dissociation curves

Fig. 1 shows the O_2Hb dissociation curves for the blood of each of the three subjects at the three CO_2 tensions of 15, 40 and 70 mm Hg. They are very similar to the curves of Barcroft & Poulton (1913) and Bock, Field & Adair (1924) showing the effect of increasing CO_2 tension on the O_2Hb dissociation curve. Where the curves of these authors diverge from one another those in

Fig. 1 lie between the two. This supports the view of Bock *et al.* (1924) that minor differences may arise from the different methods of blood gas analysis employed, since we used the Roughton–Scholander syringe method whereas Bock *et al.* (1924) employed the Van Slyke apparatus, and Barcroft & Poulton (1913) adopted the Barcroft–Haldane technique.

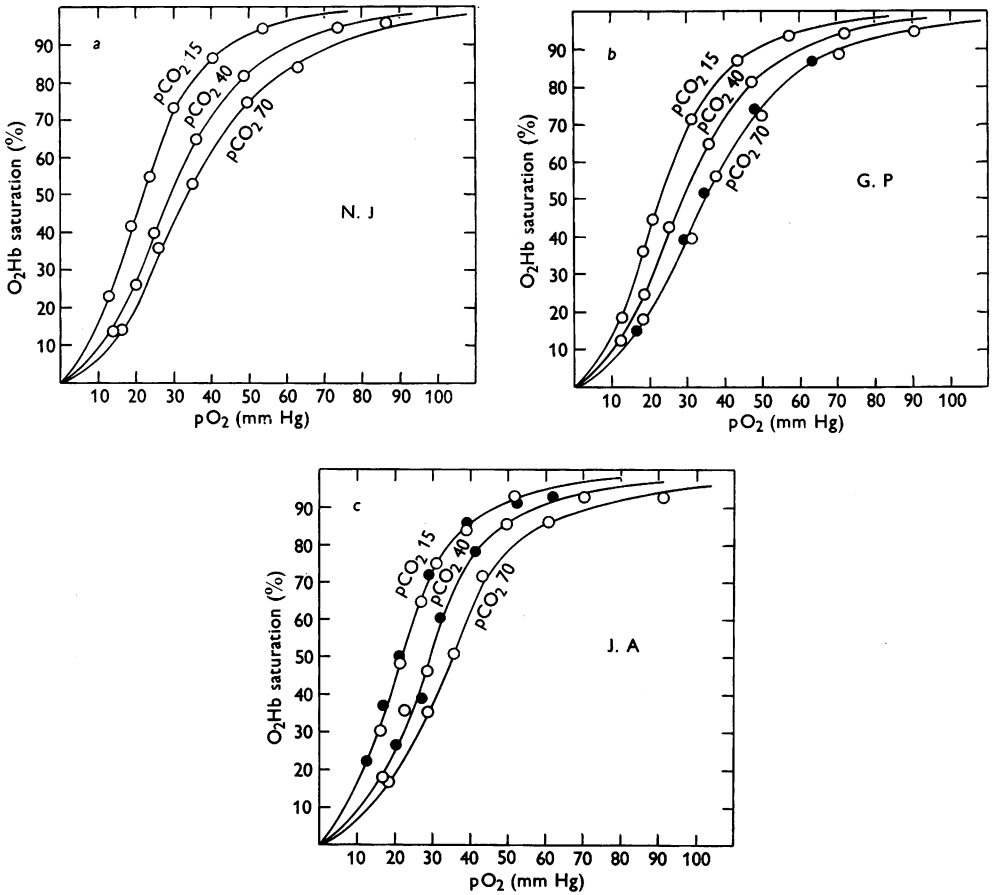

Fig. 1. O_2Hb dissociation curves for each of the three subjects at CO_2 tensions 15, 40 and 70 mm Hg, and pH values of 7·50, 7·25, and 7·15 respectively. Closed and open circles on the same curve denote results of separate experiments at the same CO_2 tension and pH value.

Individual and day-to-day variations in the curves are illustrated by Fig. 2 in which the determinations on all three subjects at each pCO_2 have been plotted on the same graph. While the number of points for each subject is probably too small for any dogmatic assertion, there does not seem to be any significant difference between the subjects. Fig. 2 also shows that when the O_2Hb dissociation curve of a given subject at a given pCO_2 was repeated, even

after an interval of several months, there was likewise little difference in the results. This contention that there is little individual or daily variation in the curves is contrary to the findings of some earlier workers, but, unlike them, we have been able to correct our points for differences in pH. Since the effect of pH is comparatively large in the range 7·2–7·6 such differences may be significant.

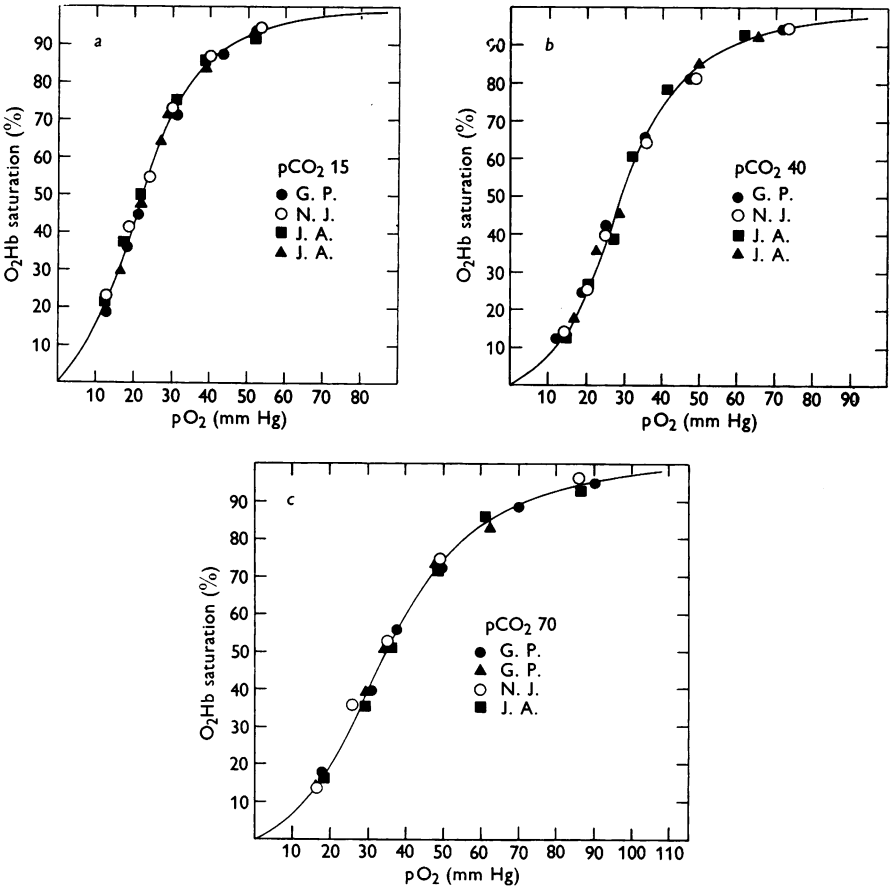

Fig. 2. O_2Hb dissociation curves drawn through the combined results for all three subjects at each CO_2 tension. Where two symbols are used for the results of one subject they denote the results of separate experiments.

Carboxyhaemoglobin dissociation curves

The COHb curves for each of the three subjects are depicted in Fig. 3 which shows that the effect of CO_2 on the COHb dissociation curves is apparently very similar to its effect on the O_2Hb dissociation curves. Fig. 4 demonstrates that, as in the case of the O_2Hb curves, there was little variation between the

subjects when all the determinations at each pCO_2 were plotted on the same graph. Similarly, there was no difference between curves determined on the same subject at an interval of several weeks. The part played by differences in pH in producing variations between the dissociation curves of the three individuals, and in the dissociation curve of the same individual as determined

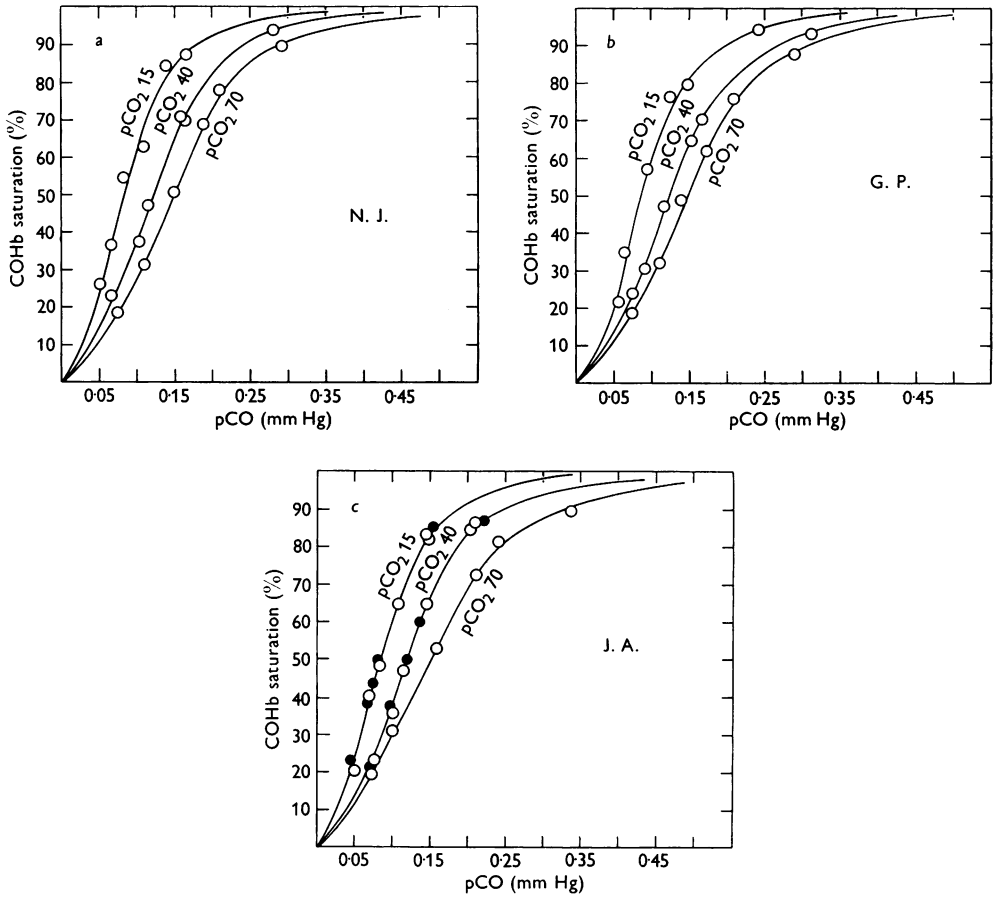

Fig. 3. COHb dissociation curves for each of the three subjects at CO_2 tensions of 15, 40 and 70 mm Hg and pH values of 7·50, 7·25 and 7·15 respectively. Closed and open circles on the same curve denote results of separate experiments at the same CO_2 tension and pH value.

on various occasions, has already been mentioned and was well illustrated by the following incident. In one experiment the COHb curve for J.A. was determined at pCO_2 40 mm. To our surprise the pH values for the equilibrated blood samples were much the same as those for the COHb curve of J.A. at pCO_2 70 mm, and on plotting the results before pH corrections had been applied the curves were found to be very similar. Questioning revealed that

on that morning J.A. had been for a vigorous swim before breakfast, followed by a brisk uphill walk of three miles to the laboratory. His blood when drawn must therefore have contained appreciable quantities of lactic acid, thus lowering the pH and accounting for these findings. The pH values confirmed this explanation which could otherwise have been only suspected.

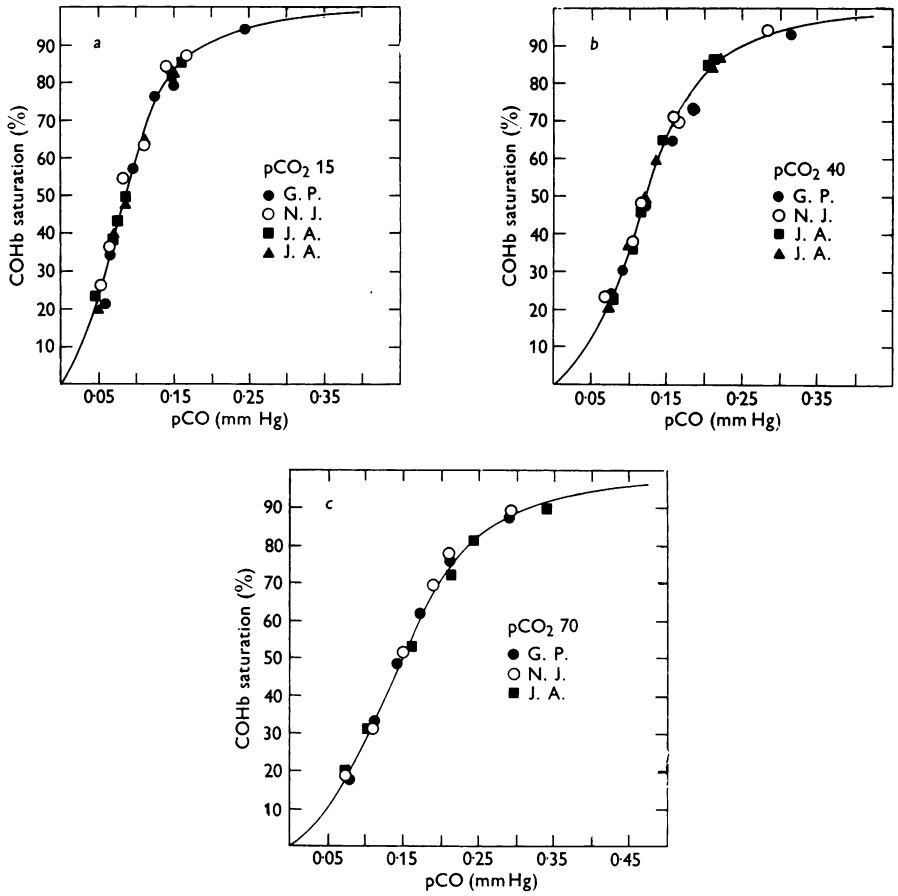

Fig. 4. COHb dissociation curves drawn through the combined results for all three subjects at each CO_2 tension. Where two symbols are used for the results of one subject they denote the results of separate experiments.

Comparison with the COHb curves of Douglas, Haldane & Haldane

The results of Douglas *et al.* (1912) are compared with those of the present investigation in Fig. 5. Although an exact comparison is not possible since the curves on the blood of Douglas were determined at CO_2 pressures of 0, 19, 42 and 79 mm, while we used CO_2 pressures of 15, 40 and 70 mm, there appears to be considerable similarity between the two sets of results. Closer examina-

tion, however, reveals that while there is good agreement over the steep middle portions of the curves, at the higher saturations our curves tend to lie above those of Douglas *et al.* (1912). In the 65–90% saturation range we have found between 2 and 5% greater saturation of the haemoglobin for any given pCO.

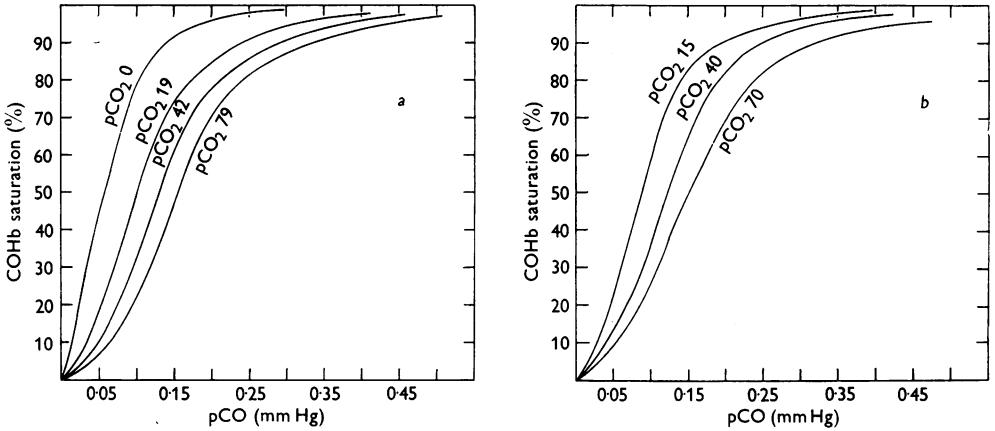

Fig. 5. (*a*) COHb dissociation curves of Douglas *et al.* (1912) for the blood of Douglas at CO_2 tensions of 0, 19, 42 and 79 mm Hg; (*b*) COHb dissociation curves drawn from the combined results of N.J., G.P. and J.A. at CO_2 tensions of 15, 40 and 70 mm Hg.

Comparison of O_2Hb and COHb curves

Douglas *et al.* (1912) noted that when allowance was made for the difference in scale of the abscissae the O_2Hb and COHb curves at any given pCO_2 were very similar. They further postulated that the COHb dissociation curves could be made to coincide over their whole range with the corresponding O_2Hb curves merely by altering the scale of the CO pressures M-fold. We have estimated the relative affinity of haemoglobin for CO and O_2, by comparing the pressures of CO and O_2, as read from our dissociation curves, at which half the haemoglobin was converted to COHb or O_2Hb, respectively. We have denoted the relative affinity as determined in this fashion by the symbol M^*, reserving the more conventional M for the relative affinity as determined from the ratio of the pressures of CO and O_2 which will convert half the haemoglobin to COHb and half to O_2Hb, reduced haemoglobin being absent. Table 1 gives the values for M^* derived from corresponding curves in Figs. 1 and 3 and also the values derived from the averaged curves of all three subjects shown in Figs. 2 and 4. Though the differences in the values for the three subjects at each pCO_2 are probably all within the range of experimental error, there is a significant increase in M^* as the pCO_2 is lowered and the blood becomes more alkaline. This increase is particularly marked when the values at pCO_2 40 mm Hg and pCO_2 15 mm Hg are compared.

In Fig. 6 the averaged O_2Hb and COHb curves of Figs. 2 and 4 have been superimposed. The CO pressures have been multiplied by the value of M^* appropriate to the particular pCO_2, thus compensating for the difference in scale of the CO and O_2 pressures. Over the middle ranges of the curves there is good agreement; at the lower ends there are variable differences but the small number of results at the lower saturations makes comment about the divergences difficult. At their upper ends, however, all the COHb curves do appear to lie about 1% below the corresponding O_2Hb curves, though this difference cannot be much greater than the error of the saturation determination.

TABLE 1. Values of M^* at each CO_2 tension obtained as described in the text from the O_2Hb and COHb dissociation curves of N.J., G.P. and J.A., together with values derived from the combined curves of Figs. 2 and 4

CO_2 tension (mm Hg)	N.J.	G.P.	J.A.	Combined curves
15	265	267	250	259
40	236	234	242	238
70	223	233	232	232

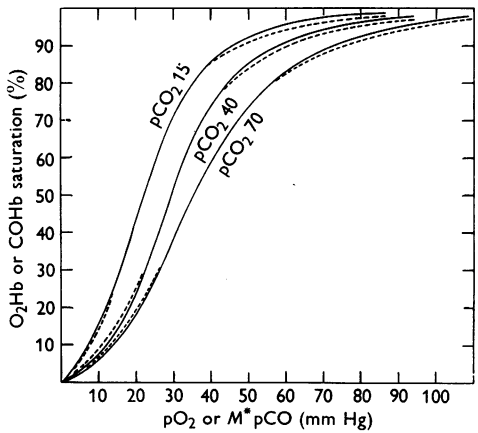

Fig. 6. Superimposed O_2Hb and COHb dissociation curves for the three subjects at CO_2 tensions of 15, 40 and 70 mm Hg and pH values 7·50, 7·25 and 7·15, respectively. At each CO_2 tension the CO pressures have been multiplied by the appropriate value of M^* (see table). The curves virtually coincide and are shown as single continuous lines except at their upper and lower ends where the digression of the COHb curves is indicated by the dotted lines.

DISCUSSION

The COHb dissociation curve

Interest in the reaction between haemoglobin and CO, in the absence of O_2, may be said to have commenced with one or two preliminary experiments by Haldane & Lorrain Smith (1897) in which they observed the saturation with COHb after shaking haemoglobin solutions with mixtures of CO and hydrogen.

It was not until 1912, however, that the first complete dissociation curves were determined by Douglas *et al.* (1912). As far as we can ascertain, apart from some unpublished experiments of Hecht, Forbes & Morgan referred to by Barcroft (1928), their work has not been repeated until the present investigation, forty-five years later. Having regard to the technical difficulties which we ourselves have experienced and the improvement in apparatus and techniques now available, those earlier curves must command great admiration. As already noted, the principal difference we have observed is that our curves lie above those of Douglas *et al.* (1912) at the higher saturations. This can probably be explained by the fact that the earlier work did not include many determinations at the higher saturations and in drawing their free-hand curves the authors must have been influenced by the shape of the current O_2Hb curves. The COHb curve for the blood of Douglas at pCO_2 40 mm Hg coincides exactly with the corresponding O_2Hb curve at pCO_2 40 mm Hg presented in the same paper of Douglas *et al.* (1912). On the other hand, our COHb curves correspond more closely in shape to our own O_2Hb curves and to the O_2Hb curves of Bock *et al.* (1924). Moreover, when the O_2Hb curve of Douglas was redetermined by Courtice & Douglas (1947) using an improved Haldane blood-gas method, the curve obtained also had the same shape as our COHb and O_2Hb curves. We therefore feel that the COHb curves presented in this paper are an improvement on those of Douglas *et al.* (1912).

The relative affinity of haemoglobin for oxygen and carbon monoxide

From their earlier work on haemoglobin solutions saturated with a mixture of O_2 and CO Haldane & Lorrain Smith (1897) concluded that the affinity of haemoglobin for CO was about 300 times greater than that for O_2. Subsequent more detailed examination of this point by Douglas *et al.* (1912) demonstrated that when the blood of Douglas was exposed to a mixture of O_2 and CO which would convert half the haemoglobin to O_2Hb and half to COHb the pressures of O_2 and CO were in the ratio 246:1. This ratio was designated by the symbol M. When these same authors compared the pressures of O_2 and CO corresponding to the 50% saturation points on the separate O_2Hb and COHb curves at pCO_2 40 mm Hg, they found the pressures to be in the ratio 235:1. At that time the difference between these two values was simply explained as due to experimental error. However, F. J. W. Roughton (personal communication) has recently observed that the relative affinities of haemoglobin for CO and O_2 as determined by these two methods are actually two quite different constants, any correspondence between them being simply a numerical coincidence. He has therefore suggested that the symbol M^* should be used to denote the relative affinity as determined from the 50% saturation points on the individual O_2Hb and COHb curves. The value of 235 given by Douglas *et al.* (1912) for M^* is very similar to our results of 235–240, also at pCO_2 40 mm Hg. On the other

hand, while we found no significant difference in M^* between the bloods of our three subjects at any given pCO_2, Douglas *et al.* (1912) found that the affinity of the blood of Haldane for CO, as represented by M, was 15% greater than that of Douglas.

Several later estimates have been made of the value of M. Sendroy, Liu & Van Slyke (1929) give average values of 210 for human blood and 179 for ox blood. Sendroy & O'Neal (1955) repeated and extended this work confirming the figure of 210 for human blood and found wide species variations ranging from 162 for sheep to 247 for the blood of the opossum. Killick (1936), using the reversion spectroscope, reported values of 233–272 for the blood of four men, results closer to those of Douglas *et al.* (1912). Though Douglas *et al.* showed that changes in temperature alter the value of M, temperature differences cannot account for these various figures since they were all obtained at 37–38° C. However, little account seems to have been taken of the possibility that alterations in pCO_2 or plasma pH might also affect M. It is true that neither Douglas *et al.* (1912) nor Hartridge (1912) could find any change in M as a result of altering the pCO_2 or of adding lactic acid and Na_2CO_3 to the blood. Nevertheless, Roughton (1954), studying dilute solutions of sheep haemoglobin at 19° C, has recently found that M rose by an average of 35% when the pH was increased from 7·1 to 9·1. This change in M is at least similar in direction and degree to the results for M^* presented in this paper, which indicate that in the physiological range of pH and at 37° C there is a small, but definite increase in the relative affinity for CO as the pCO_2 is reduced and the pH increases. Allen & Root (1957) have also found M to be influenced by plasma pH, though these authors record a peak value close to 225 for the blood of men and dogs at pH 7·35 and 37° C, falling sharply above and below this pH value to as low as 140 at pH 7·6 and to 155 at pH 7·1. One consequence of the fall described by the present authors in the value of M^* as the pH is reduced is that the COHb and O_2Hb dissociation curves are not, as is generally believed, affected to the same extent by changes in pH and pCO_2. A rise in pCO_2 and fall in pH would appear, from the results presented in this paper, to produce a slightly greater shift to the right of the COHb curve than of the O_2Hb curve.

*The difference between the relative affinities M and M**

The intermediate compound hypothesis of Adair (1925) represents the reaction between haemoglobin and O_2 or CO as taking place in four stages giving a series of compounds Hb_4, $Hb_4(O_2)$, $Hb_4(O_2)_2$, $Hb_4(O_2)_3$, $Hb_4(O_2)_4$, and Hb_4, $Hb_4(CO)$, $Hb_4(CO)_2$, $Hb_4(CO)_3$, and $Hb_4(CO)_4$. The respective equilibrium constants of the various reactions have been designated K_1, K_2, K_3, and K_4 for the union with O_2 and L_1, L_2, L_3, and L_4 for the union with CO.

As stated before (see Methods), when blood is equilibrated with a mixture

of CO and O_2 at pressures sufficient to ensure that there is less than 0·1% of reduced haemoglobin present, the proportion of COHb to O_2Hb is given by the equation $\frac{(COHb)}{(O_2Hb)} = \frac{M pCO}{pO_2}$. This equation has been termed Haldane's first principle. The experimental validity of this principle has been confirmed by several workers (e.g. Allen & Root, 1957). Roughton (1954) has given mathematical proof that if the above equation is true for all values of pCO and pO_2, then $M = L_4/K_4$. Thus, at percentage saturations sufficiently high for virtually all the haemoglobin molecules to be combined with at least three molecules of O_2 or CO—that is, above 99·9% saturation—the O_2Hb and COHb dissociation curves should coincide if the scale of gas pressures is altered M-fold.

Haldane also formulated a second principle, that the dissociation curve of O_2Hb can be made to coincide with the dissociation curve of COHb over the whole saturation range by altering the scale of gas pressures M-fold. However, if Haldane's second principle is to hold then not merely must $L_4/K_4 = M$ but it is further required that $L_3/K_3 = L_2/K_2 = L_1/K_1 = M$, since the equilibrium constants L_1, L_2, L_3 and K_1, K_2, K_3 become of increasing significance as the concentrations of reduced haemoglobin and of intermediate compounds containing one or two gas molecules rise. Roughton (1954) has tested Haldane's second principle using an ingenious and delicate technique to study the upper ends of the COHb and O_2Hb dissociation curves in the range 98–99·5% saturation. He found that while at pH 9·1 the two curves could be made to coincide by multiplying the pCO values by M, at pH 7·1 MpCO was about 2·5 times pO_2, so that Haldane's second principle did not hold true in this saturation range at physiological pH. The discrepancy could be explained by supposing that L_1/K_1, L_2/K_2 and L_3/K_3 were not equal to M. This supposition has since been confirmed by Roughton, Otis & Lyster (1955) who measured the individual equilibrium constants of the intermediate reactions between O_2 and haemoglobin at pH 9·1 and 19° C and found K_4 to be 18 times greater than K_1. Similar measurements by Roughton (1954) showed L_4 to be 50 times greater than L_1. Thus, if as has been shown $L_4/K_4 = M$, then $L_1/K_1 = 0{\cdot}36M$.

The failure of Haldane's second principle resulting from this alteration in the relative affinity of haemoglobin for O_2 and CO with varying degrees of saturation makes quite clear the difference between M as originally defined by Haldane in the absence of reduced haemoglobin, and our determinations of M^* where 50% of the haemoglobin was in the reduced form. This variation in the relative affinity with the degree of saturation no doubt explains the divergences which have been observed between the COHb and O_2Hb dissociation curves at their upper and lower ends when the CO pressures were multiplied by M^* or M. Had it not been for the close numerical similarity of M and M^* the elucidation of these divergences between the curves would not

have had to await the very precise measurements of saturation developed by Roughton and his colleagues during the past decade.

It therefore appears that temperature and species are only some of the factors affecting the relative affinity of haemoglobin for O_2 and CO. Not only are both CO_2 pressure and plasma pH of undoubted significance, but the degree of saturation of the haemoglobin must also be taken into account.

SUMMARY

1. Carboxyhaemoglobin dissociation curves, in the absence of O_2, have been prepared for the blood of three subjects at CO_2 pressures of 15, 40 and 70 mm Hg, and pH values of 7·50, 7·25 and 7·15, respectively. The determinations were made by equilibrating blood samples in tonometers with various concentrations of CO and measuring both the COHb saturation of the blood and the CO concentration in the tonometer gas at equilibrium.

2. These curves have been compared with the O_2Hb dissociation curves of the same three subjects, in the absence of CO, at the same CO_2 pressures and pH values. The effect of CO_2 on the COHb dissociation curves is shown to be very similar to though not identical with its effect on the O_2Hb dissociation curves.

3. The COHb dissociation curves differ slightly from the curves published by Douglas *et al.* (1912). Reasons for the differences are given.

4. The relative affinity of haemoglobin for CO and O_2 varies only slightly from subject to subject. On the other hand, it is affected by changes in plasma pH and CO_2 pressure, the relative affinity for CO rising as the pH increases and the CO_2 pressure falls. In these experiments, which were performed at 37° C, the ratio of the CO pressure producing 50% saturation with COHb to the O_2 pressure producing 50% saturation with O_2Hb rose from an average value of 1:230 at pH 7·15 and pCO_2 70 mm Hg, to 1:260 at pH 7·50 and pCO_2 15 mm Hg.

5. The relative affinity as determined in this fashion from the 50% saturation pressures on the individual COHb and O_2Hb dissociation curves has been termed M^*. The differences between this value and M, the ratio of the CO and O_2 pressures producing 50% COHb and 50% O_2Hb in a mixture containing no reduced haemoglobin, are discussed.

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