THE EFFECT OF CARBON MONOXIDE ON THE OXYHEMOGLOBIN DISSOCIATION CURVE

F. J. W. ROUGHTON AND R. C. DARLING

From The Fatigue Laboratory, Harvard University, Boston, Massachusetts

Received for publication October 29, 1943

J. B. S. Haldane (13) was the first to study theoretically the equilibrium between hemoglobin and mixtures of O₂ and CO which were of insufficient concentration to saturate the hemoglobin completely. His treatment was subsequently amplified and modified by A. V. Hill (15), Stadie and Martin (23), Barcroft (4), and Peters and Van Slyke (19). As a result of this work it has been generally accepted that partial saturation of the blood hemoglobin with CO shifts the oxygen dissociation curve of the remaining hemoglobin progressively to the left, and also makes the curve less S-shaped and more hyperbolic ("Haldane effect"). This indicates that the blood in vivo, if partially saturated with CO, must cling to its O₂ with greater tenacity so that the tissues have much more difficulty in obtaining O₂ from the blood than they do when the O₂Hb of the blood is reduced to a corresponding extent by anemia. This deduction has been used by Haldane to explain why "Miners may do their ordinary work though their hemoglobin percentage is reduced to half by ankylostomiasis—whereas a person whose blood is half saturated with CO is practically helpless."

A somewhat puzzling and paradoxical feature of CO-poisoning which has been explained by the Haldane effect is that with mice at very low pO₂, the addition of a trace of CO to the inspired air may actually help the organism to acquire O₂.

Haldane’s original theory was only tested experimentally by observations on the CO-dissociation curve of hemoglobin when part of the hemoglobin was combined with O₂; parallel observations as to the effect of partial combination of the hemoglobin with CO on the O₂ dissociation curve of the remaining hemoglobin were not available until the work of Stadie and Martin twelve years later. These authors considered that their hypothesis was well supported by what they thought was excellent agreement between theory and experiment. Doctor Stadie in a recent conversation has, however, permitted us to say that in his present opinion the agreement was not as good as could be desired, particularly as the observations were limited to a somewhat narrow portion of the dissociation curve.

Since the subject is of interest, alike in the theory of the hemoglobin reactions and in the practical study of the effects of CO poisoning both at sea level and high altitudes, we have thought it desirable to reinvestigate the subject in a more complete fashion. In so doing we have been led to some profitable simplifications in the development of the theory. It is satisfactory to find that the original

1 Partial saturation of the hemoglobin with O₂ has an exactly similar effect on the CO-dissociation curve of the residual hemoglobin.
Haldane theory, as thus extended, has stood up well to our more searching tests and we feel that it can therefore be used with confidence in more detailed calculations as to the toxicity of CO especially at high altitudes. The latter problem has been considered, as regards its importance in aviation medicine, by Heim (14).

Review of the Haldane theory and description of simplified method of application. When blood or hemoglobin is brought into equilibrium with gas mixtures containing O₂ and CO at pressures such that the amount of reduced hemoglobin is negligible, the proportion of CO-hemoglobin to O₂-hemoglobin is generally agreed to follow the equation:

\[
\frac{[\text{COHb}]}{[\text{O₂Hb}]} = \frac{M_p\text{CO}}{p\text{O₂}}
\]

The value of \( M \) is independent of pH, salts and dilution of the blood, but varies with temperature, illumination and species. There is some dispute as to whether \( M \) varies from individual to individual in a given species; thus Douglas, Haldane and Haldane (11) with their carmine titration method of estimating COHb, found values of \( M \) ranging from 220 to 290 in man, whilst Killick (17) with the reversion spectroscope reported figures of 230 to 270 in man. Sendroy, Liu and Van Slyke (22), however, claim that for six men \( M \) is constant at 210 to ±2.5 per cent, and for ten different ox bloods is constant at 179 (±2.5 per cent). These figures are for a temperature of 37 to 38°C.

In addition to these factors there is also the question of the presence of pseudo-hemoglobin in the blood. According to Barkan (5) normal blood contains small amounts of two pigments, pseudohemoglobin and pseudomethemoglobin, which are intermediate in composition between ordinary hemoglobin and bile pigments. These pigments are distinguished and estimated by the ease with which metallic iron can be split from them in acid solution. Barkan deduces, by an indirect method, that the value of \( M \) for pseudohemoglobin is around 10 times greater than that for normal hemoglobin.

If the sum of the \( p\text{O₂} \) and \( p\text{CO} \) is not sufficient to saturate the hemoglobin and there is an appreciable amount of reduced hemoglobin present, the amounts of COHb and O₂Hb at equilibrium can be worked out from the O₂-dissociation curve in absence of CO if Haldane’s two basal assumptions are made:

A. That the amount of reduced hemoglobin present in a mixture of O₂ at partial pressure \( p\text{O₂} \) and of CO at partial pressure \( p\text{CO} \), is the same as it would be in absence of CO if the partial pressure of O₂ was equal to \( p\text{O₂} + M_p\text{CO} \), \( M \) being defined as in equation 1. The amount of reduced hemoglobin can thus be read off from the O₂-dissociation curve in absence of CO.

B. That the hemoglobin combined with gas is partitioned between COHb and O₂Hb according to equation 1 even when there is appreciable reduced hemoglobin present.

The method of calculation now to be described embodies features drawn from previous treatments especially that of J. B. S. Haldane but is simpler and involves fewer assumptions than any of them. It may be most readily explained by working through a typical example.
Let figure 1, curve A, represent the dissociation curve of blood in absence of CO but at pCO₂ = 40 mm., pH = 7.4, 37°C.

It is required to find the pO₂ in equilibrium with the blood when [COHb] = 20 per cent, [O₂Hb] = 32 per cent, [Reduced Hb] = 48 per cent of the total hemoglobin.

We have [COHb] + [O₂Hb] = 20 + 32 = 52 per cent of the total hemoglobin.

From figure 1A it is seen that the gas pressure corresponding to 52 per cent = 27.4 mm.

Therefore:

\[ pO₂ + MpCO = 27.4 \text{ mm.} = pO₂ \left(1 + \frac{MpCO}{pO₂}\right) \quad (2a) \]

Now by equation (1):

\[ \frac{MpCO}{pO₂} = \frac{[COHb]}{[O₂Hb]} = \frac{20}{32} \quad (2b) \]

Therefore:

\[ pO₂ + MpCO = pO₂ \left(1 + \frac{[COHb]}{[O₂Hb]}\right) = pO₂ \left(1 + \frac{20}{32}\right) = 27.4 \quad (2c) \]

So \( pO₂ = 16.9 \text{ mm.} \)

It will be seen that the calculated value of pO₂ is entirely independent of the values of M and pCO₂, which are in fact irrelevant. This result is made use of in the experimental section and is further considered in the discussion.

Since

\[ 100 \times \text{per cent } O₂Hb/(\text{per cent } O₂Hb + \text{per cent Red. Hb}) = \]

\[ 100 \times \frac{32}{32 + 48} = 40 \text{ per cent} \]
by plotting 40 against 16.9, we obtain a point on the O₂-dissociation curve of the remaining 80 per cent of the blood hemoglobin which is not combined with CO, i.e., Y of figure 1B.

Similarly for \([\text{COHb}] = 20\) per cent, \([\text{O₂Hb}] = 56\) per cent, \([\text{Red. Hb}] = 24\) per cent of the total hemoglobin we have

\[
pO_2 = \frac{41.2 \text{mm.}}{(1 + \frac{100}{56})} = 30.4 \text{ mm. and } 100 \times \frac{\text{per cent O₂Hb}}{\text{per cent O₂Hb} + \text{per cent Red. Hb}} = 100 \times \frac{56}{56 + 24} = 70 \text{ per cent}
\]

thus obtaining a second point on the O₂ dissociation curve at constant COHb concentration = 20 per cent, i.e., X on figure 1B.

Repetition of this procedure for other selected values of the per cent O₂Hb enables the whole O₂-dissociation curve at 20 per cent COHb to be plotted as in figure 1, curve B.

Exactly the same method of calculation can then be applied for COHb percentages, e.g., of 40 per cent, 60 per cent (fig. 1, curves C and D) or of any other desired value. From the empiric O₂-dissociation curve in absence of CO we thus readily derive a family of curves relating pO₂ to \([\text{O₂Hb}]/(\text{O₂Hb} + \text{Red. Hb})\) at selected constant values of [COHb], without making any assumption at all as to the equation of the O₂-dissociation curve.

Our method therefore seems preferable to most previous treatments since in these the validity of Hill's equation for the dissociation curve is assumed. This equation now has no theoretical basis and can only be used as an empirical expression over the middle of the curve, but not at the extremes.

EXPERIMENTAL. A. Hemoglobin solutions. The following data were taken from our earlier work (9) on hemoglobin solution in which the shifts in the O₂-dissociation curves due to COHb and methemoglobin were compared, but the shift due to COHb was not analyzed at that time in relation to the Haldane theory.

Cleared human hemoglobin solutions in 0.6 M phosphate solution, pH 7.4 at 37°C were used. The mixtures of COHb and O₂Hb were made by first equilibrating a portion of the solution with CO (tension = approx. 50 mm. Hg) and then mixing this solution with O₂Hb in the desired proportion. The mixture was then divided into several portions and rotated with various mixtures of O₂ and N₂. After this second equilibration of one or two hours, the liquid phases were analyzed for O₂ and CO and the gas phases for O₂. It was found that negligible CO had been lost from the liquid phase during this equilibration. It is certain that one to two hours' rotation were enough to bring the O₂ in the gas phase and the liquid phase into equilibrium with one another and with the hemoglobin. It is also most probable that equilibrium was complete between the hemoglobin and the dissolved CO, since at 37°C. Roughton's (20) observations show that the half-time of chemical dissociation of COHb is only a few seconds. On the other hand, owing to the low pressure gradient of CO between the blood and gas phases it seems equally clear (from calculations like those of Roughton (21) on the rate of gas-liquid exchange in manometric reactions) that it would take many hours for diffusion to bring the CO in the gas phase to its final equi-
librium value. This latter lack of equilibrium would, however, only matter if
the theory required an exact knowledge of the equilibrium pCO of the gas phase;
this, as pointed out above, is not the case—all the theory requires is that the
equilibrium in the blood between the Hb, O₂ and CO should be complete and that
the per cent O₂Hb, per cent COHb, and equilibrium pO₂ should be known. These
factors are given by the blood-gas analyses. Technically it is a great advantage
not to have to continue the tonometer equilibration for many hours, since pos-
sibility of decomposition of the hemoglobin, and, in the case of whole blood, of
pronounced glycolysis, is thereby reduced.

Figure 2 presents a summary of these data. The three curves reading from
right to left are, A, the measured dissociation curve of O₂Hb in the absence of
CO; B, the curve calculated according to theory for dissociation of O₂Hb in the
presence of 23.5 per cent COHb, and C, the same type of calculated curve in the
presence of 32 per cent COHb. The solid circles (●) represent actual experi-
mental points of a solution containing 23.5 per cent COHb. The test of the
theory is the closeness with which these points approach curve B. Similarly
the open circles (○) represent experiments on a solution containing 32 per cent
COHb and should lie on curve C, if the theory is correct.

It will be seen that four of the six experimental points lie on the theoretical
curve well within the experimental error. The other two points do not exactly
fit the theoretical curve but are not sufficiently distant to rule out some technical
error, especially since these experiments were not checked at the time to test
this point.

B. Human blood. Blood of a single subject was used throughout in four
experiments. It was drawn in the morning from an antecubital vein, mixed
with heparin and placed in the icebox, from which portions were removed when
needed. Fresh blood was drawn for each day’s experiments.

A careful O₂ dissociation curve of each sample of drawn blood was determined
with special attention to the upper half of the curve (from which the curves
for partial conversion to COHb are chiefly calculated). In the first three ex-
periments the tonometers for equilibration were made up with a pCO₂ of 40 mm.
Hg, in the fourth with pCO₂ = 25 (in addition to the desired pO₂).

Although the subject did not smoke for 12 hours prior to bleeding, there was
always found a small per cent of COHb (4 to 5.5 per cent) in his blood. On
figure 3 the curves of his blood are corrected to 0 per cent COHb according to
the theory in the introduction, and from these curves the theoretical curves are
calculated for the desired per cent COHb. It may be noted that even at the
same pH there is slight day to day variation in the position of the curve; thus
it was important to determine the curve at 0 per cent COHb for each day’s ex-
periment.

The experimental equilibrium points at the chosen per cent of COHb were
determined in a manner similar to that used for hemoglobin solutions. A portion
of the blood was equilibrated with N₂ containing CO to a pressure of 50 mm.
Appropriate mixtures of this CO blood were made in a syringe with some of the
original blood and stored in the ice-box for several hours. This allows time for
diffusion to bring about a uniform distribution of the CO between the red cells, which were initially saturated with the gas, and those which at the start were practically CO-free. The mixture was then equilibrated in a series of tonometers made up with varying O$_2$ pressures and a pCO$_2$ equal to that used on the 0 per cent COHb curve of that day. The equilibrium time at 37° was 30 minutes. As with the hemoglobin solutions the CO of the blood was found not to diffuse into the gas phase to an appreciable extent. On theoretical grounds one should again expect equilibrium within the liquid phase but not between liquid and gas in this time. For purposes of testing the theory the latter as shown above is not necessary.

Experiments 1, 2 and 3 were done with a pCO$_2$ of 40, thus giving a pH of somewhat lower than 7.4 and approximating blood conditions in vivo at sea level. For experiment 4 a pCO$_2$ of 25 was chosen to approximate the extreme conditions in vivo with hyperpnea such as would occur at high altitudes.

All blood samples were analyzed for CO$_2$, O$_2$ and CO by the methods of Van Slyke-Neill with some modifications of Horvath and Roughton (16). Tonometer gases were analyzed for O$_2$ and CO$_2$. From these data serum CO$_2$ and pH$_s$ were calculated for each blood sample using the line chart of Peters and Van Slyke and the Henderson-Hasselbalch equation. The mean pH of the CO-containing bloods was taken as standard for the experiment. Among these CO-containing bloods the pH$_s$ never varied more than 0.02 from the mean, but the points on the curve of the blood as drawn were usually slightly less acid (due to shorter equilibration and therefore less glycolysis). The curve of 0 per cent COHb usually needed correction to reach the chosen standard pH$_s$; for this the empirical relationship of Dill et al. (10) was used ($\Delta$ log. pO$_2$ = $-0.48\Delta$ pH). For example, the observed value for one of the points with 0 per cent COHb in experiment 3 was 60.5 per cent O$_2$Hb at pO$_2$ = 34.4 and pH$_s$ = 7.42. For correction to pH$_s$ = 7.36, $\Delta$ pH$_s$ = -0.06. Therefore, $\Delta$ log pO$_2$ = $-0.48(-0.06) = +0.029$, and corrected pO$_2$ = antilog (log 34.4 + 0.029) = 36.0.

Figure 3 presents the results of the four experiments, presented similarly to figure 2. The solid lines on the right in each graph present the measured O$_2$ dissociation curve of the CO free blood. The one or two solid lines on the left are the theoretical curves calculated from the right hand curve. The points plotted are the actually observed values of the CO-containing bloods. As before the test of the theory is the closeness of these points to the corresponding theoretical curve.

As with the hemoglobin solutions, it will be seen that the great majority of the points lie close to or on the theoretical curves. Only two points out of a total of 26 (both in expt. 2) are clearly distant from the curves. We have no explanation for these discrepancies, but attribute them to undetected errors in our procedures, since the agreement is generally good in experiment 3, which is a repetition of experiment 2. Dissociation curve experimentation of this kind is in fact a somewhat exacting task, especially when done in hot and humid weather as were these experiments; in previous work it has not been uncommon for one or two points out of ten to be distinctly out of line with the remainder, due presumably to human fallibility.
Discussion. **Physico-chemical.** The treatment based on the assumptions A and B (v. review of Haldane theory on p. 18) was shown above to lead to deduction of the effect of a given per cent COHb without any assumption as to the numerical value either of M or of pCO at equilibrium. Independence of M indicates that the COHb effect would not be changed either by any of the factors on which M depends, namely, temperature, illumination, species, or even in mixtures of two pigments of different M values, as may occur in blood when appreciable amounts of pseudohemoglobin (Barkan) are present. The independence of pCO has already proved very advantageous in the experimental procedure. The whole treatment is thus more simple and comprehensive than those based on pCO and numerical value of M (e.g., Stadie and Martin, Van Slyke, Barcroft); the only disadvantage is that it does not inform us of the values of pCO at equilibrium. These, however, are not necessary for testing the theory nor are they of much physiological importance since it is rare for a subject to go on breathing a CO-containing atmosphere long enough to reach complete equilibrium.

So important are assumptions A and B for the whole problem that it is necessary to inquire how they stand in regard to the physico-chemical theories of the O₂-hemoglobin equilibrium. Their relation to two of the older theories has already been considered by Haldane and A. V. Hill. The subsequent measurements of the osmotic pressure and molecular weight of hemoglobin by Adair...
(1, 2) and by Svedberg (24) have, however, shown that the main postulates both of the Haldane and the Hill theory are invalid; the only offshoot of either of these theories which still survives in the literature is Hill’s equation for the $O_2$-hemoglobin equilibrium, this being retained and used only on account of its convenience as an empirical expression. On the other hand, wide acceptance has been and is still given to the intermediate compound hypothesis, first put forward in general form by Adair (1, 2) and subsequently developed along a special and very fertile line by Pauling (18). According to Adair’s hypothesis the reaction between $O_2$ and hemoglobin takes place in four stages:

\[
\begin{align*}
O_2 + Hb_4 & \rightleftharpoons Hb_4O_2, \quad [Hb_4O_2] = K_1 [O_2] [Hb_4] = K_1 p z \\
O_2 + Hb_4O_2 & \rightleftharpoons Hb_4O_4, \quad [Hb_4O_4] = K_2 [O_2] [Hb_4O_2] - K_1 K_2 p^2 z \\
O_2 + Hb_4O_4 & \rightleftharpoons Hb_4O_6, \quad [Hb_4O_6] = K_3 [O_2] [Hb_4O_4] = K_1 K_2 K_3 p^3 z \\
O_2 + Hb_4O_6 & \rightleftharpoons Hb_4O_8, \quad [Hb_4O_8] = K_4 [O_2] [Hb_4O_6] = K_1 K_2 K_3 K_4 p^4 z
\end{align*}
\]

where $[O_2] = p$, $[Hb_4] = z$ and $K_1$, $K_2$, $K_3$, $K_4$ are the equilibrium constants of the respective reactions. Similarly the reaction of CO with hemoglobin takes place in four stages: $CO + Hb_4 \rightleftharpoons Hb_4CO$ (equilibrium constant $L_1$), $CO + Hb_4CO \rightleftharpoons Hb_4(CO)_2$ (equilibrium constant $L_2$), etc. The fact that the dissociation curve of oxyhemoglobin agrees exactly with that of carboxyhemoglobin if the scale of gas pressures is altered $M$-fold is most simply explained by assuming $L_1/K_1 = L_2/K_2 = L_3/K_3 = L_4/K_4 = M$. Roughton (20) has applied the intermediate compound hypothesis to the equilibrium between CO, $O_2$ and Hb when the pressures of the two gases are such as to saturate the hemoglobin almost completely: no previous attempt has been made, so far as we know, to handle, on the basis of the intermediate compound hypothesis, the equilibrium between CO, $O_2$ and Hb when appreciable amounts of reduced hemoglobin are present, as in the experimental work of this paper. We shall now show that assumptions A and B fit in readily with the intermediate compound hypothesis, if a few additional and reasonable assumptions be made.

Consider first the intermediate compounds in which only one of the four gas-combining spaces of the hemoglobin molecules is occupied, $Hb_4O_2$ and $Hb_4(CO)$. Let $[Hb_4] = z$, $[O_2] = p$, $[CO] = q$

Then $[Hb_4O_2] = K_1 p z$ and $[Hb_4(CO)] = L_1 q z$

From this it follows that the ratio of combined CO to combined $O_2$ is $\frac{[Hb_4(CO)]}{[Hb_4O_2]} = L_1 q / K_1 p = \frac{M q}{p}$, i.e., assumption B holds.

Also the total number of molecules of combined gas ($O_2 + CO$) is $[Hb_4O_2] + [Hb_4(CO)] = (K_1 p + L_1 q) z = (K_1 p + K_1 q L_1 / K_1) z = K_1 (p + M q) z$, which according to equation (3) is the same as the number of molecules of $O_2$ which would be so combined, if no CO was present but the $O_2$ pressure was equal to $(p + M q)$, i.e., assumption A holds.

Next consider the intermediates containing 2 molecules of gas, i.e., $Hb_4O_4$, $Hb_4O_2 \ (CO)$, $Hb_4(CO)_2$. 
We then have

\[ \text{Hb}_4\text{O}_4 = K_1 K_2 p^2 z \]
\[ \text{Hb}_4\text{CO}_2 = L_1 L_2 q^2 z \]
\[ \text{Hb}_2\text{CO}_2 = K_3 L_1' p q z \]

where \( L_2 \) is the equilibrium constant of the reaction \( \text{CO} + \text{Hb}_4\text{O}_2 \rightleftharpoons \text{Hb}_4\text{O}_2\text{(CO)} \), and \( L_1' \) is the velocity constant of the reaction \( \text{Hb}_4\text{O}_2 + \text{CO} \rightarrow \text{Hb}_4\text{O}_2\text{(CO)} \) = velocity constant of reaction \( \text{Hb}_4\text{O}_2\text{(CO)} \rightarrow \text{CO} + \text{Hb}_4\text{O}_2 \).

We now introduce the new assumptions to which we have already referred. Let us assume that the velocity constant of the reaction \( \text{CO} + \text{Hb}_4\text{O}_2 \rightarrow \text{Hb}_4\text{O}_2\text{(CO)} \) is the same as that of the reaction \( \text{CO} + \text{Hb}_4\text{O}_2 \rightarrow \text{Hb}_4\text{O}_2\text{(CO)} \), i.e., the chance of CO combining with a molecule of hemoglobin in which one of the four spaces is already occupied is the same whether that one space is occupied by \( \text{O}_2 \) or \( \text{CO} \); furthermore, assume on the same general grounds that the velocity constant for the dissociation reaction \( \text{Hb}_4\text{O}_2\text{(CO)} \rightarrow \text{Hb}_4\text{O}_2 + \text{CO} \) is half that of the dissociation reaction \( \text{Hb}_4\text{O}_2\text{(CO)} \rightarrow \text{Hb}_4\text{CO} + \text{CO} \) since in the latter case there are two CO molecules available to dissociate whereas in the former there is only one.

If these assumptions are accepted \( L_2' = 2L_2 \)

The ratio of combined CO radicals to combined \( \text{O}_2 \) radicals in the intermediates containing two molecules of gas then

\[ \frac{\text{[Hb}_4\text{O}_2\text{(CO)}] + 2\text{[Hb}_4\text{O}_2\text{(CO)}_2]}{2\text{[Hb}_4\text{O}_2\text{(CO)}] + \text{[Hb}_4\text{O}_2\text{(CO)}_2]} = \frac{K_1 L_2 p q z + 2L_1 L_2 q z}{2K_1 K_2 p^2 z + K_1 L_2 p q z} \]

\[ = \frac{2K_1 L_2 p q z + 2L_1 L_2 q z}{2K_1 K_2 p^2 z + 2K_1 L_2 p q z} = \frac{K_1 L_2 q (p + L_2 q / K_1)}{K_1 K_2 (p + L_2 q / K_2)} \]

\[ = \frac{K_1 L_2 q (p + M q)}{K_1 K_2 (p + M q)} = \frac{M q}{p} \]

i.e., assumption B holds for the 2-molecule containing intermediates as well as for the 1-molecule containing intermediates.

The total number of gas molecules combined in the 2-molecule intermediate form

\[ = 2\text{[Hb}_4\text{O}_4] + 2\text{[Hb}_4\text{O}_2\text{(CO)}] + 2\text{[Hb}_4\text{(CO)}_2] = 2K_1 K_2 p^2 z + 2K_1 L_2 p q z + 2L_1 L_2 q z \]

\[ = 2K_1 K_2 p^2 z + 4K_1 L_2 p q z + 2L_1 L_2 q^2 z = 2K_1 K_2 z (p^2 + 2p q / K_2 + q^2 / K_2) \]

\[ = 2K_1 K_2 (p^2 + 2M p q + M q^2) = 2K_1 K_2 (p + M q)^2 z \]

Thus the total number of gas molecules combined both in the 1-molecule and the 2-molecule containing intermediate = \( K_1 (p + M q) z + 2K_1 K_2 (p + M q)^2 z \). This according to equations (3) and (4) is the same as the number of molecules of \( \text{O}_2 \) which could be so combined if no CO was present and the \( \text{O}_2 \) pressure was equal to \( (p + M q) \), i.e., assumption A again holds.
The same arguments can in turn be applied to the 3-molecule and 4-molecule containing intermediates: the calculation process becomes progressively more complicated but the end result is the same, namely, the maintenance of assumptions A and B. Thus with relatively few and reasonable auxiliary assumptions the two main postulates on which the calculation of the O2-hemoglobin dissociation curve in presence of CO is based, can be reconciled with the general form of the intermediate compound hypothesis proposed by Adair. The same result is reached with Pauling’s special form of the intermediate compound hypothesis if similar reasoning and auxiliary assumptions are used, so the detailed working out in this case may be left to the reader who wishes to undertake it. It may be noted that Pauling’s interaction constant, $\alpha$, must be assumed to remain the same whether the neighboring molecule which exerts its interaction effect is O2 or CO.

In a recent paper we have shown that partial conversion to methemoglobin also shifts the O2-dissociation curve of the remaining curve to the left. In five out of eleven cases the shift was almost exactly equal to that found with the same amount of COHb, but in the other six cases the shift was only about half as great. The causes of this variability have not yet been worked out, but at all events we feel convinced that the explanation of the methemoglobin effect must be qualitatively of the same type as that of the COHb effect. Further support for this is given by our observation that the two effects were additive. Conant and Fieser (6) suggested that the equilibrium between O2, O2Hb, MetHb, ferri-cyanide and ferrocyanide could be expressed by the equation:

$$\frac{[\text{MetHb}]}{[\text{O2Hb}]} = \frac{N[\text{ferri-cyanide}]}{p\text{O}_2[\text{ferrocyanide}]}$$

Equation (7) is analogous to equation (1), where N is an equilibrium constant and if similar assumptions to (A) and (B) hold good in the case of MetHb, the effect of the latter on the O2-dissociation curve should be calculable by equations (2a), (2b) and (2c) with $\text{MP}CO$ replaced by $N[\text{ferri-cyanide}]/[\text{ferrocyanide}]$ and $[\text{COHb}]/[\text{O2Hb}]$ by $[\text{MetHb}]/[\text{O2Hb}]$. The actual shift of the dissociation curve would thus only depend on the fraction $[\text{MetHb}]/[\text{O2Hb}]$ and not on N, [ferri-cyanide] and [ferrocyanide] except insofar as these determine $[\text{MetHb}]/[\text{O2Hb}]$. It should furthermore be the same for a given [MetHb] as for the same [COHb], as indeed we found in 5 out of 11 cases.

The actual values of N, as calculated by Conant and Fieser from a series of experiments in which Hb was treated with various amounts of ferri-cyanide and equilibrated with O2, show, however, a rather wide scatter. In subsequent data by Conant and Scott (7) on the corresponding equilibrium between CO, COHb, MetHb, ferri-cyanide and ferrocyanide, no actual calculations of the equilibrium constant are quoted by the authors. The figures given by them are, however, adequate for this purpose and we have worked out the value of the equilibrium constant $= p\text{CO}[\text{MetHb}]/[\text{ferrocyanide}]/[\text{COHb}][\text{ferri-cyanide}]$ from their experimental data on solutions equilibrated with pure CO. The values of the constant unfortunately show a scatter of at least 10-fold. It therefore seems scarcely
safe at present to apply any quantitative theory on this basis to the effect of MetHb on the $O_2$-dissociation curve.

**Physiological Discussion.** In subjects exposed to carbon monoxide, two important problems arise. 1. How fast does the per cent COHb in the blood increase? 2. What is the effect of a given per cent COHb in the blood on the loading and unloading of $O_2$? The rate and extent of the rise of per cent COHb depends upon the pCO, pO$_2$ in the air breathed, the time of exposure and the ventilation rate. These factors have already been investigated in other laboratories and are to be dealt with more fully in forthcoming papers from this laboratory. We shall here concern ourselves with problem 2.

To begin with we must establish that the effect of COHb on the $O_2$-dissociation curve as determined in vitro in the experimental section also applies quantitatively in circulating blood during gas exchange in the capillaries. When there is no COHb in the blood, the $O_2$-dissociation curve of the blood as determined in vitro is generally assumed to apply also in vivo, since the rate of exchange of $O_2$ between chemical combination with Hb and physical solution in the red cell and plasma is known to be so fast. The presence of appreciable COHb introduces two possible complications: 1, that its amount might change during the passage of the blood through the capillary, and 2, that the distribution of CO between chemical combination and physical solution might not be fast enough for the equilibrium state to be preserved throughout the capillary phase. In regard to the first point the diffusion pressure of dissolved CO in the blood is so low that it is unlikely that any gain or loss in total CO of the blood could occur during a single passage of blood through the tissue capillary except perhaps in the case of muscle, where there is possibility of very rapid combination with the appreciable amounts of myoglobin contained therein. That the average change for all the capillaries of the body is not significant is shown by the observation that in the whole animal the per cent COHb in the blood after exposure to CO normally takes several hours to drop to half its value. As regards the second point it is true that the unloading of $O_2$ in the capillary leads to an increased concentration of reduced hemoglobin in the blood which in turn will cause transfer of a minute amount of CO from physical solution to combination with Hb. Suppose in a typical instance that blood entering the capillary contains 30 per cent COHb, 67 per cent $O_2$Hb, pO$_3$ = 100 mm. Hg, pCO = 0.21 mm. Hg, and leaves the capillary with 35 per cent $O_2$Hb. Reference to figure 1 and to equations (2a), (2b) and (2c) shows that the pO$_3$ in the venous blood will be 19 mm. and the pCO = 0.08 mm. The drop in pCO will thus only amount to 0.13 mm. and the corresponding rise in per cent COHb will be only about 0.002. From Roughton's data it can be calculated that the time for such a change is about 0.01 sec., which is 1/100 or less than the average time spent by the blood in the capillary. At each instant during passage of blood through the capillary the CO as well as the $O_2$ is therefore effectively in equilibrium between chemical combination and physical solution. As regards $O_2$ distribution the condition of the blood accordingly traverses the $O_2$-dissociation corresponding to the assigned [COHb] of the blood. As regards CO, the [COHb] remains effectively constant whereas the
pCO traverses a course which cannot be measured directly but can be calculated as in the example just given. All these considerations make it evident that the evaluation of the shift of the \( O_2 \)-dissociation curve in terms of the COHb content is much more apposite from the physiological viewpoint as well as more convenient from the experimental angle than is its evaluation in terms of pCO.

The smaller amount of hemoglobin available for \( O_2 \) transport in CO poisoning together with the shift of the dissociation curve of the residual Hb to the left (cf. fig. 1) were considered by Haldane as additive factors in explaining the special handicap to the organism in \( O_2 \) unloading. Actually, however, the recent observations of Asmussen and Chiodi (3) do not indicate as severe a handicap as might be expected from this line of approach. Thus at rest, at light work and at heavy work 33 per cent COHb in the blood led to no appreciable change in the \( O_2 \) consumption per minute nor was any change found in the cardiac output, increase in which might have compensated for the adverse effect of change in the dissociation curve. In a later paper (8) such compensation was, however, shown to appear

---

**Fig. 4.** Calculated \( O_2 \)-dissociation curves of human blood containing varying amounts of carboxyhemoglobin, plotting the absolute amounts of bound \( O_2 \) rather than the percentage of available hemoglobin bound to \( O_2 \).
CARBON MONOXIDE AND OXYHEMOGLOBIN DISSOCIATION

in resting subjects when the per cent COHb exceeded 40. For these reasons we feel that the alternative method of plotting given in figure 4 (and independently used by Dr. J. R. Pappenheimer) is more appropriate for physiological use than that adopted by Haldane. Here the total amount of bound O$_2$ is plotted against pO$_2$ instead of the amount relative to the available hemoglobin as in figure 1. It will be seen that the curves for the various percentages of COHb are widely different at the higher O$_2$ pressures but are almost coincident at the lower pressures.

In normal man at rest at sea level only the upper half of the steep part of the O$_2$-dissociation curve is made use of in O$_2$ unloading, the lower half (i.e., 10–25 mm. pO$_2$) of the steep part being kept as a reserve, which is only called upon in exercise or in pathological conditions. In the presence of COHb it seems clear that this reserve could be called upon if the shape and portion of the O$_2$-dissociation curve as plotted in figure 4 in the 10 to 25 mm. pO$_2$ range was about the same as in the absence of COHb. Figure 4 shows that the dissociation curves over this low range are very much the same for all per cent COHb within 0 to 40, and therefore the O$_2$ uptake can be kept steady just by using up more or even all of the reserve normally available from the shape of the dissociation curve. Above 40 per cent COHb figure 4 shows that the type of reserve given by the dissociation curve rapidly becomes exhausted and it is therefore not surprising that a dangerous situation rapidly develops. The carotid sinus is generally supposed to be sensitive to the pO$_2$ rather than to the O$_2$ content of the arterial blood, and since in CO poisoning at sea level there is no change in arterial pO$_2$, there is no stimulus of the sinus to provide compensatory increase in heart output or ventilation, as there is when breathing air of low pO$_2$. The lack of such compensation, together with depletion of reserve, explains the sudden collapse at rest if the per cent COHb is increased above the critical level of 40 to 50 per cent COHb. Resting subjects at or even below this critical level frequently collapse if they take mild exercise: the reason for this is that the reserve, though adequate in rest, is no longer big enough to supply the increased need of the body in work.

The curve for 40 per cent anemia is also presented in figure 4 to demonstrate again the marked difference as regards O$_2$ unloading from that which obtains when 60 per cent of the Hb is combined with CO rather than merely absent. The anemic subject has the further advantage of lowered blood viscosity, which may lead in turn to an earlier and more pronounced compensatory increase in circulation rate than that found in CO-poisoning.

Figure 4 is also specially useful in another connection. At very low O$_2$ pressures Haldane and L. Smith (12) made the startling observation that mice might be actually benefited by the addition of small amounts of CO in the inspired air. Haldane explained this in terms of the appreciable increase in O$_2$ which the blood would take up when exposed to a low loading O$_2$ tension in the presence of COHb, owing to the shift to the left in the dissociation curve produced by the latter. We must also, however, consider how much O$_2$ remains bound to the Hb at the unloading tension at the venous end of the capillary, since no benefit will result to the organism unless the difference between the bound O$_2$ at the beginning and
end of the capillary is increased. Thus with an arterial $pO_2$ of 20 mm. and a venous $pO_2$ of 12 mm., the $O_2$ unloaded is, (v. fig. 4) 3.5 vols. per cent in the case of CO-free blood, is only 3.2 vols. per cent in the case of blood containing 20 per cent COHb. On the other hand the $O_2$ unloaded between an arterial $pO_2$ of 12 mm. and a venous pressure of 4 mm. is 2.7 vols per cent for CO-free blood and 2.9 vols. per cent in the case of 20 per cent CO-blood. At pressures above 20 mm. the unloading from CO-free blood is progressively better than from CO-containing blood; it is only in the region of the lower inflection of the dissociation curve that the opposite effect is seen to occur. Since pressures in the neighborhood of 12 mm. are lower than man can tolerate, it does not appear possible that the Haldane-Smith effect could ever be observed in man. In the case of mice, however, the $O_2$-dissociation curve of the blood is shifted greatly to the right of man, the affinity being only about one-third in this region: the critical $O_2$ tension at which a beneficial effect of CO might occur would therefore be expected to be about 3 times greater, namely, at 36 mm. Haldane's observations have shown that mice in absence of CO can withstand $O_2$ pressures in the inspired air as low as 36 mm., and so in this species the Haldane-Smith effect should manifest itself quite definitely. From this method of attack it will be seen that whether or not any given species of animal will benefit from small amounts of CO when exposed to very low $O_2$ pressures will depend both on the position of the $O_2$-dissociation curve and on the critical value of $O_2$ pressure in the inspired air at which brain function fails, i.e., the oxygen ceiling.

CONCLUSIONS

1. A simplified method is proposed for calculating the $O_2$-dissociation curve of $O_2Hb$ in the presence of a given per cent of COHb. It utilizes the observed dissociation curve of $O_2Hb$ without CO and the theoretical assumptions of the partition of Hb between $O_2$ and CO, but avoids any equation for the hemoglobin dissociation curve and does not require the choice of a numerical value for the partition coefficient M.

2. The theory was tested in hemoglobin solutions in 0.6 M phosphate buffer pH 7.4 at two percentages of COHb and found to agree excellently with four out of six observed points, and the discrepancy in the remaining two was not very serious.

3. Whole blood showed generally excellent agreement between theory and observation when similarly tested at pCO$_2$ = 40 per cent and COHb approximately 20, 40, 60 per cent, and at pCO$_2$ = 25; per cent COHb = 22.

4. The relation of the fundamental assumptions of the theory to the intermediate compound hypothesis of the $O_2$-hemoglobin equilibrium is worked out.

5. It is shown that the effect of COHb on the $O_2$-dissociation curve in vivo should be quantitatively the same as the experimentally observed effect in vitro.

6. Plotting of $O_2$ pressure against total bound $O_2$ rather than against the fraction of the available hemoglobin bound with $O_2$ is shown to give clearer indications of the effect of COHb on the transport of $O_2$. In particular the conditions
under which $a$, $O_2$ supply would begin to fail, and $b$, small amounts of CO would have a beneficial effect at very low $O_2$ pressures (Haldane-Smith effect) are demonstrated.

REFERENCES

(1) ADAIR, G. S. J. Biol. Chem. 63: 529, 1925.
(3) ASMUSSEN, E. AND H. CHIODI. This Journal 132: 426, 1941.
(9) DARLING, R. C. AND F. J. W. ROUGHTON. This Journal 137: 56, 1942.
(11) DOUGLAS, C. G., J. S. HALDANE AND J. B. S. HALDANE. J. Physiol. 44: 275, 1912.
(22) SENDROY, J., S. H. LEE AND D. D. VAN SLYKE. This Journal 90: 511, 1920.