

ON THE COMBINATION OF NITRIC OXIDE WITH
HÆMOGLOBIN. BY M. L. ANSON AND A. E. MIRSKY¹.

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IN the course of some investigations on the relative affinities of oxygen and carbon monoxide for hæmoglobin it was desired to have a third gas that combines loosely with hæmoglobin. It has long been known that nitric oxide reacts with hæmoglobin and that it can displace carbon monoxide from its combination with hæmoglobin. It has been supposed that the reaction between NO and hæmoglobin is of the same nature as that between CO and hæmoglobin; that NO displaces CO from its combination with hæmoglobin for the same reason that CO displaces O₂.

We found that in the reaction between NO and HbO₂ or HbCO the first step is the formation of methæmoglobin. (The anomalous results with reduced hæmoglobin will not be considered here.) A solution of HbO₂ or HbCO is injected by means of a syringe into a tonometer containing NO diluted with hydrogen. The events are followed spectroscopically. If the solution is kept alkaline, alkaline methæmoglobin first appears, and this then reacts with NO to form what is ordinarily known as NOHb, but which should be called NOMHb because it is with methæmoglobin that NO combines. Previous workers have always kept the solution alkaline to prevent it from becoming too acid as a result of possible nitrous acid formation. Not wishing the solution to become too acid they never had the opportunity of observing what occurs in even faintly acid solution. If, however, the experiment is repeated in slightly acid medium, acid methæmoglobin first appears and NO reacts with this to form a new compound whose absorption spectrum and colour are different from those of alkaline NOMHb. The new form is much browner than the alkaline form and its two-banded spectrum is more distinct. The α band is at 5687 Å. In a few minutes, however, the new form goes over into the alkaline form of NOMHb. If this solution is now made still more acid, this transformation is reversed and the new form reappears. In a solution that is acid enough to begin with, the alkaline form never appears at all. After finding out that the first step

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in the reaction is the formation of methæmoglobin we always began the experiments with methæmoglobin itself.

Methæmoglobin is an indicator (1, 2). At pH 9 methæmoglobin exists entirely in the alkaline form with its characteristic colour and spectrum; at pH 8 it is entirely in the acid form, the colour and spectrum of which are entirely different from those of the alkaline form, and at intermediate H-ion concentrations there are various mixtures of these two forms. If the pH is plotted against the percentage composition of these mixtures as determined spectroscopically, the curve obtained is the characteristic curve of the influence of pH on the dissociation of a weak acid. Alkaline methæmoglobin, then, is simply the ionised and acid methæmoglobin, the un-ionised form of a weak acid whose dissociation constant (actually about $10^{-8.5}$) is given by the turning point of methæmoglobin as an indicator.

NOMHb also proves to be an indicator with a turning point at about pH 5.6. In other words NOMHb is of the order of a thousand times as strong an acid as is methæmoglobin. This explains why at certain H-ion concentrations although acid NOMHb is formed it changes over into the alkaline form. The NO combines with un-ionised methæmoglobin to give un-ionised NOMHb which at that pH proceeds to dissociate. If the acid NOMHb is to be permanent, the solution must be much more acid than is necessary merely to have methæmoglobin in the un-ionised form; it must be acid enough to assure that the NOMHb formed will remain completely un-ionised.

The hydrogen-ion concentration influences not only the spectrum of methæmoglobin but also its affinity for NO. The NO of alkaline NOMHb cannot be removed with the pump. The NO of acid NOMHb, on the contrary, can be pumped off easily. The [H] is known to have a similar influence on the affinity of hæmoglobin for oxygen, and this effect has been interpreted as meaning that oxyhæmoglobin is a stronger acid than is reduced hæmoglobin. The same interpretation applies to the analogous NOMHb case. The relations between the equilibrium and the dissociation constants are given by the mass law equations which apply generally to dilute solutions.

$$\begin{aligned}
 (1) \quad \frac{[H^+][MHb^-]}{[HMHb]} &= K_1. & (2) \quad \frac{[H^+][NOMHb^-]}{[NOHMHb]} &= K_2. \\
 (3) \quad \frac{[NO][MHb^-]}{[NOMHb^-]} &= C_1. & (4) \quad \frac{[NO][HMHb]}{[NOHMHb]} &= C_2. \\
 \frac{(1)}{(2)} &= \frac{(3)}{(4)} \quad \text{or} \quad (5) \quad \frac{K_1}{K_2} = \frac{C_1}{C_2}.
 \end{aligned}$$

The exact affinities of acid and alkaline methæmoglobin for NO have not been measured, but their approximate values can be estimated by comparison with the known affinities of hæmoglobin for O₂ and CO. Alkaline methæmoglobin has a much *greater* affinity for NO than Hb has for CO and acid methæmoglobin has much *less* affinity for NO than hæmoglobin has for O₂. Since hæmoglobin has about four hundred times greater affinity for CO than for O₂, $\frac{K_1}{K_2}$ must be of the order of a thousand. It follows from equation (5) that NOMHb is about a thousand times as strong an acid as is methæmoglobin. This value agrees with the estimate already made *independently* from the spectroscopic data.

In contrast, oxyhæmoglobin is only 25 times(3) as strong an acid as reduced hæmoglobin. In certain hæmocyanins, however, the difference between the dissociation constants of the oxygenated and reduced forms appears to be of the same magnitude as the difference between the dissociation constants of NOMHb and MHb. In these hæmocyanins (at body temperatures) the oxygen can be set free only if the solution is made acid, say by bubbling CO₂ through it(4).

SUMMARY.

1. Nitric oxide forms a loose combination with methæmoglobin.
2. Nitric oxide methæmoglobin is about a thousand times as strong an acid as is methæmoglobin.

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