Porphyrin Studies: Kinetics of Metalloporphyrin Formation[1]

By EVERLY B. FLEISCHER, E. I. CHOI, PETER HAMBRIGHT, and ALLEN STONE

A mechanism for the formation of metalloporphyrins in aqueous solutions is formulated for tetrapyridylporphine.   The order of reactivity of metals toward metalloporphyrin formation is Cu(II) > Zn(II) > Mn(II) > Co(II) > Fe(II) > Ni(II) > Cd(II).   The equilibrium constant for the equilibrium between tetrapyridylporphine, metal ion, and the porphyrin sitting-atop complex is measured for various ions.

Previous studies have dealt with the kinetic and equilibrium properties of the porphyrin molecule.[2-9]

The reaction of metal ions with the porphyrin nucleus to form metalloporphyrin complexes was investigated with the purpose of determining the mechanism of the reaction and the order of reactivity of various metal ions.   The over-all reaction is given in eq. 1.[10]

$$\text{TPyPH}_2 + \text{M(H}_2\text{O)}_6{}^{2+} \rightarrow$$
$$\text{M-TPyP(H}_2\text{O)}_2 + 2\text{H}^+ + 4\text{H}_2\text{O} \quad (1)$$
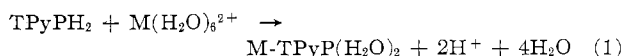
Results

The rates of formation of metalloporphyrins were studied in the pH range 1.5–2.4.   In this pH range tetrapyridylporphine exists predominantly in the free base form.[12]

The sitting-atop complex of tetrapyridylporphine is formed in the range of metal concentrations used in this study.[4,11]   The spectra that clearly demonstrate the existence of a porphyrin species that is not either the free base or diacid porphyrin are shown in Fig. 1.   Curve 1 is the spectrum of tetrapyridylporphine free base at a pH of 2.2.   Curve 2 is the spectrum of the tetrapyridylporphine diacid in 1.0 M HNO$_3$.   Curve 3 is the spectrum of tetrapyridylporphine with sodium ions at a pH of 2.2 and 4.0 M sodium nitrate.   The spectra of other sitting-atop complexes of tetrapyridylporphine in aqueous solution are similar to the sodium spectrum.   The spectra of a series of solutions of constant porphyrin and varying sodium ion show isosbestic points at 418, 498, and 536 mμ.   The Soret region, from 380 to 480 mμ, of such a series of varying sodium ion concentrations from 0 to 4.0 M with con-

stant concentration of tetrapyridylporphine of 4.5 $\times$ 10^{-6} M is shown in Fig. 2.   A titration of tetrapyridylporphine with acid gives a set of isosbestic points at 420 and 560 mμ.   The fact that the spectra of the free base and the diacid, and the free base and the sitting-atop species, exhibit different sets of isosbestic points shows that there is indeed a third species of porphyrin that exists at pH 2.0 in the presence of metal ions that is not either the free base or diacid species.[13]   It has also been previously shown that the monoprotonated species of tetrapyridylporphine is not formed in aqueous solutions.[4]

Since the rate of formation of the sitting-atop complexes is very fast compared to the metalloporphyrin formation, it is possible to measure the equilibrium constant for the formation of the sitting-atop species.

Since this equilibrium will effect the kinetics of the metalloporphyrin formation it is important to know the K_{sat} for various metals.

The color of the porphyrin free base is red, of the sitting-atop complex of TPyP green, and of the metalloporphyrin orange.   This change in color makes both the equilibrium constants and the kinetics easy to follow spectrophotometrically.

Let $D_0{}^\lambda$ stand for the initial optical density of a solution of TPyPH$_2$ at wave length λ, $D_\infty{}^\lambda$ the limiting value of the optical density at high metal ion concentration, and $D_x{}^\lambda$ the optical density of a solution of TPyPH$_2$ with metal ion concentration x, where in general the optical density D^λ is given by

$$D^\lambda = \epsilon_i{}^\lambda C_i l$$

where $\epsilon_i{}^\lambda$ is the extinction coefficient of the ith species, C_i is the concentration of the ith species, and l is the path length of the cell in cm.   We will set $l = 1.0$ cm.   By determining a set of spectra of TPyPH$_2$ at constant total porphyrin, varying the metal ion concentration for each solution, and making the following assumptions we can derive an expression for the equilibrium constant.[14]   (1) The only absorbing species are the

(1) This work was supported by a Public Health Service Grant and by the Louis Block Fund.

(2) E. B. Fleischer, *Inorg. Chem.*, **1**, 493 (1962).

(3) E. I. Choi and E. B. Fleischer, *ibid.*, **2**, 94 (1963).

(4) E. B. Fleischer and L. E. Webb, *J. Phys. Chem.*, **67**, 1131 (1963).

(5) M. B. Lowe and J. N. Phillips, *Nature*, **190**, 262 (1960).

(6) W. S. Caughey and A. H. Corwin, *J. Am. Chem. Soc.*, **77**, 1509 (1955).

(7) A. H. Corwin and P. Wei, *J. Org. Chem.*, **27**, 4285 (1962).

(8) A. H. Corwin and R. Singh, *ibid.*, **28**, 2476 (1963).

(9) J. N. Phillips, *Rev. Pure Appl. Chem.*, **10**, 35 (1960).

(10) The following conventions are used in this paper. TPyPH$_2$ stands for α,β,γ,δ-tetra(4-pyridyl)porphine and will be called tetrapyridylporphine. TPyPH$_4{}^{2+}$ stands for the tetrapyridylporphine diacid.   M-TPyP stands for the tetrapyridylporphine metal complex.   M-SAT-TPyPH$_2{}^{2+}$ stands for the tetrapyridylporphine sitting-atop complex.[11]   The charges refer only to the porphyrin nucleus and not to the molecule as a whole.   The tetrapyridylporphine in acid aqueous solution always has four charges due to the protons that are on the pyridyl groups.

(11) E. B. Fleischer and J. H. Wang, *J. Am. Chem. Soc.*, **82**, 3498 (1960).

(12) At a pH of 2.0 the amount of diacid in a solution 1.0 $\times$ 10^{-5} M in porphyrin is 1.8 $\times$ 10^{-7} M, or less than 2% of the total porphyrin exists in the diacid form.   (Calculated from equilibrium constant in ref. 4.)

(13) We prefer to call this species of porphyrin a sitting-atop porphyrin because of previous evidence that the metal ion is above the plane of the porphyrin and not in the plane like the metal in a metalloporphyrin.[11]   It is not at all clear at this time what the nature of the bonding is in these sitting-atop complexes and thus the name π-complex which has been proposed is not appropriate at this time because we do not know if the complex is of the π-type.   We only know it is a species of porphyrin different from the free base, diacid, and metalloporphyrin.

(14) The K_{sat} values are not real equilibrium constants because we are assuming that the activity coefficients are unity.   Thus we are dealing with concentration quotients.
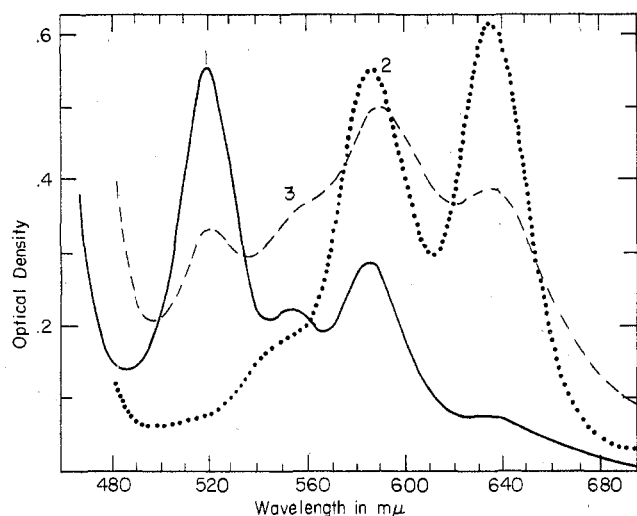
Fig. 1.—Absorption spectra of 4.2×10^{-6} M solutions of tetra-pyridylporphine in 10.0-cm. cells at 25°: 1, TPyPH₂ at pH 2.2; 2, TPyPH₂ at pH 0.0 (1 M HNO₃); 3, TPyPH₂ at pH 2.0 with 4 M NaNO₃.

free base porphyrin and the sitting-atop complex; (2) Beer's law is obeyed in the range of concentrations studied.

The above assumptions hold very well. It has been shown that Beer's law is obeyed with TPyPH₂ in aqueous solutions.[2,4] The isosbestic points of the absorption spectra of TPyP with various metal ion concentrations demonstrate that there are only two independent absorbing species in the solution. Let C_t be the total porphyrin concentration, C_{fb} be the porphyrin free base concentration, C_{sat} be the porphyrin sitting-atop concentration, ϵ_{fb}^λ be the extinction coefficient of the porphyrin free base at wave length λ, and ϵ_{sat}^λ be the extinction coefficient of the porphyrin sitting-atop species. Then

$$x\mathrm{M}^{+m} + \mathrm{TPyPH_2} \rightleftarrows (\mathrm{M})_x\text{-SAT-TPyPH}_2{}^{+mx} \quad (2)$$
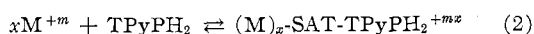
$$K_{sat} = \frac{C_{sat}}{C_m{}^x C_{fb}} \quad (3)$$

$$\log \frac{C_{sat}}{C_{fb}} = \log K_{sat} + x \log C_m \quad (4)$$

and since

$$C_t = C_{sat} + C_{fb}$$

then

$$D_0{}^\lambda = \epsilon_{fb}{}^\lambda C_{fb} = \epsilon_{fb}{}^\lambda C_t$$

$$D_\infty{}^\lambda = \epsilon_{sat}{}^\lambda C_{sat} = \epsilon_{sat}{}^\lambda C_t$$

$$D_x{}^\lambda = \epsilon_{fb}{}^\lambda C_{fb} + \epsilon_{sat}{}^\lambda C_{sat}$$

$$\frac{D_0{}^\lambda - D_x{}^\lambda}{D_x{}^\lambda - D_\infty{}^\lambda} = \frac{C_{sat}}{C_{fb}} \quad (5)$$

Thus a plot of $\log [D_0{}^\lambda - D_x{}^\lambda]/[D_x{}^\lambda - D_\infty{}^\lambda]$ vs. $\log [C_m]$ will give a straight line with the log $[D_0{}^\lambda - D_x{}^\lambda]/[D_x{}^\lambda - D_\infty{}^\lambda] = 0$ intercept of $-\log K$ and slope of x, the number of metal ions per porphyrin molecule in the sitting-atop complex. Figure 2 shows a typical
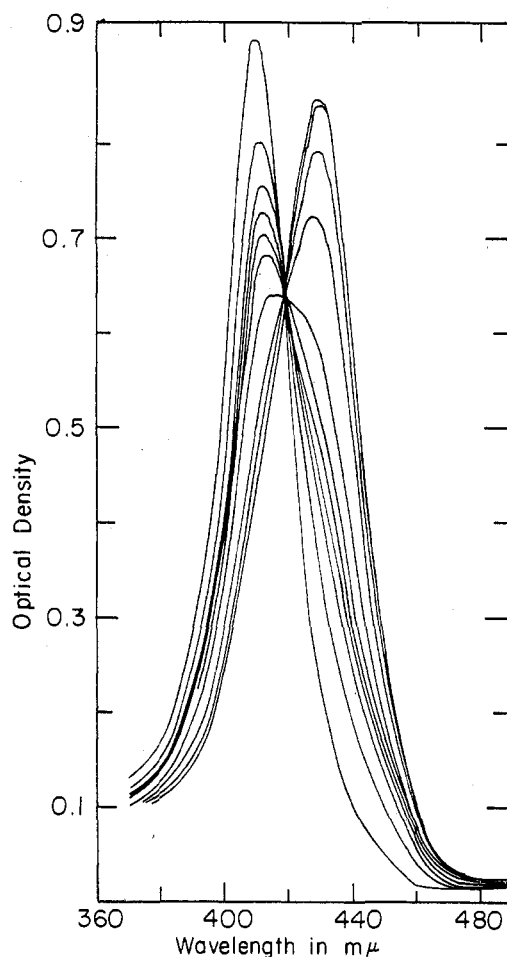

Fig. 2.—Absorption spectra of a series of solutions at pH 2.0 of constant TPyPH₂ concentration, 4.5×10^{-6} M, and varying sodium ion concentration. The sodium ion concentration ranges from 0 to 4.0 M. The peak at 410 mμ is that of the porphyrin free base while the peak at 430 mμ is that of the sitting-atop complex.

set of spectra for a series of solutions of constant total porphyrin concentration and varying sodium ion; Fig. 3 shows a plot of these data employed to determine the equilibrium constants.[14] From this type of plot the equilibrium constants for various ions were determined and are listed in Table I. The range of

TABLE I

EQUILIBRIUM CONSTANT K_{sat} FOR THE SITTING-ATOP COMPLEX, 25°, pH 2.0[a,b]

Ion[d]	K_{sat}[c]	Ion	K_{sat}
K⁺	1.5 ± 0.1	Mn²⁺	9.8 ± 0.3
Na⁺	2.3 ± 0.1	Mg²⁺	9.8 ± 0.3
(CH₃)₄N⁺	4.4 ± 0.1	Co²⁺	15.8 ± 0.5
Cd²⁺	4.9 ± 0.2	Ni²⁺	19.0 ± 0.5
Li⁺	5.4 ± 0.2	(C₂H₅)₄N⁺	26.3 ± 0.4
NH₄⁺	6.2 ± 0.2	Al³⁺	53.7 ± 0.7
Fe²⁺	6.9 ± 0.2	Cr³⁺	77.5 ± 0.5
Zn²⁺	7.7 ± 0.2	Fe³⁺	84.7 ± 0.7[e]
Ca²⁺	7.6 ± 0.2	Cu²⁺	86.9 ± 0.5

[a] Anions present were all nitrates. [b] pH fixed with nitric acid. [c] Errors are estimated from fit of the straight line in the graphs and from estimated errors in metal ion concentration. [d] The metal ion concentration range was from zero to 5.0 M or until the spectra showed a definite leveling off. [e] Corrected for hydrolysis.
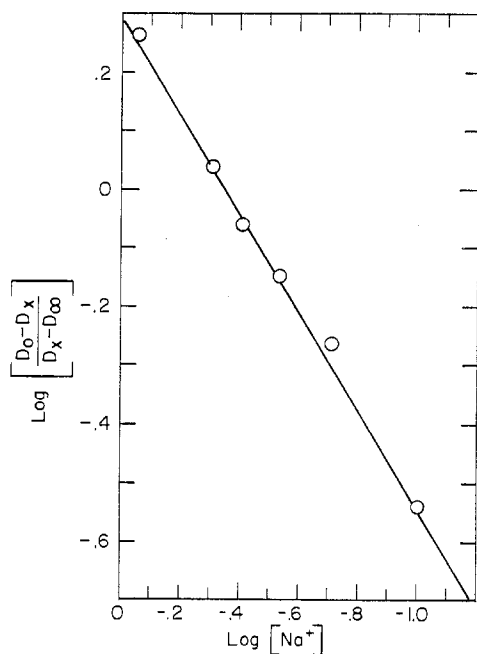
Fig. 3.—A typical plot for the determination of equilibrium constants, K_{sat}. The above is TPyPH$_2$ at pH 2.0 with sodium ions. The plot has a slope of 1.1 and an intercept of -0.36.

Fig. 4.—A plot of the kinetic data for the formation of zinc tetra-pyridylporphine at pH 2.0 and 25.0°.

values of x was from 0.85 to 1.10. This clearly indicates that in all the ions listed there is a one to one ratio of porphyrin to metal ion in the sitting-atop complex.

Kinetics of Metalloporphyrin Formation.—The rate of formation of metalloporphyrins was followed as a function of metal ion, pH, and metal ion concentration. The reactions were all run under pseudo-first-order conditions, *i.e.*, with an excess of metal ion over the tetrapyridylporphine. The optical density of the solution of metal ion and TPyPH$_2$ was observed as a function of time. If the log $(D_t - D_\infty)$ at wave length λ is plotted against time, a pseudo-first-order rate constant k_{obsd} is obtained. D_t is the optical density of the solution at time t and D_∞ is the optical density of the solution at the end of the reaction.

The reaction was followed in a pH range of 1.4–2.4 and the metal ion concentration ranges were 0.05 to 1.3 M. A typical plot of the rate data for ZnTPyP formation at a constant pH of 2.0 is given in Fig. 4. Runs were carried out at various pH values and a plot of $1/k_{obsd}$ *vs.* hydrogen ion activity is a straight line.

TABLE II

RATE CONSTANTS FOR METALLOPORPHYRIN FORMATION

Ion	$k = k_1 K_{sat}'$, sec.$^{-1}$
Zn^{2+}	1.5×10^{-4} [a]
Cd^{2+}	6.9×10^{-7}
Cu^{2+}	2.3×10^{-3}
Ni^{2+}	1.1×10^{-6}
Co^{2+}	5.9×10^{-6}
Mn^{2+}	2.3×10^{-5}
Fe^{2+}	2.7×10^{-6}

[a] The errors in the rate constants are difficult to estimate but from the average deviations of the pseudo-first-order constants and the errors in the equilibrium constants involved in getting the k we believe the data are good to at least 15–20%. Each rate constant was determined from at least 20 runs at various metal ion concentrations and pH values.
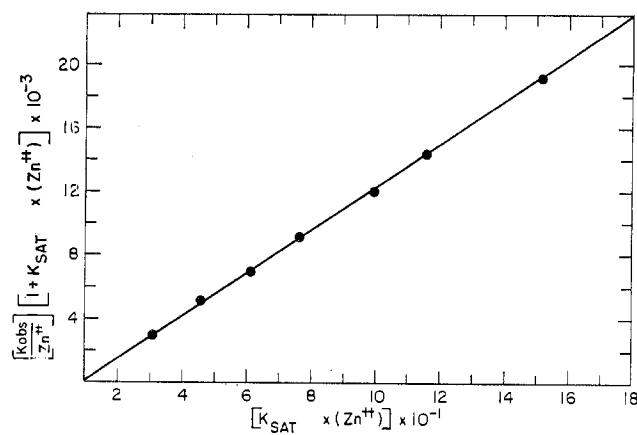
The rate constants obtained for various metals are given in Table II. The anion present in these kinetic studies was nitrate. The pH was adjusted with nitric acid and measured both before and after the reaction was carried out.

Discussion

Equilibrium Constants for the Sitting-Atop Complexes.—Tetrapyridylporphine forms a sitting-atop complex with metal ions in aqueous solution. The equilibrium studies were all carried out with nitrate as the anion. It has previously been found that sitting-atop complexes form in nonaqueous solvents for a variety of porphyrins.[11,15]

It has not been possible to identify definitely the sitting-atop complexes in aqueous solutions for porphyrins other than the tetrapyridylporphine. Either a precipitate forms on addition of the metal to the porphyrin solution or else there is not definite spectroscopic evidence for the sitting-atop formation. Since most water-soluble porphyrins that we investigated had carboxyl groups on them, it might be that the carboxyl groups prevent the sitting-atop formation. This could happen *via* two paths. Either the carboxyl group will coordinate with any metal ion that approaches the porphyrin, thus preventing the sitting-atop complex from forming, or else there is the possibility that the carboxyl group is hydrogen bonded to the pyrrole nitrogens, thus blocking any sitting-atop formation.
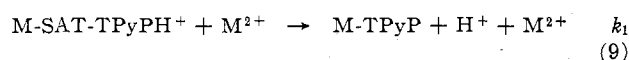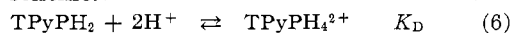
Table I shows TPyPH$_2$ to be an almost universal ligand for positively-charged ions. The most surprising ions that form the sitting-atop complex are the ammonium, tetramethylammonium, and tetraethylammonium ions. The order of stability of the complexes in Table I seems to follow a charge–radius type of correlation. It seems inappropriate to attempt any quantitative correlation of the data in Table I.

The fact that the K_{sat} decreases on going from ammonium to tetramethylammonium is reasonable on a size argument, but the large increase of stability of the tetraethylammonium ion is anomalous. It might be

(15) E. B. Fleischer, Ph.D. Dissertation, Yale University, 1961.

due to the possibility of hydrophobic interaction between the ethyl groups and the porphyrin ring. It is of interest to note that $Cr(H_2O)_6^{3+}$ forms a sitting-atop complex whose stability is in line with other 3+ ions. Since the $Cr(H_2O)_6^{3+}$ must have its coordination sphere intact[16] in the complex, this indicates that the sitting-atop complexes might have their metal–water coordination sphere intact in these sitting-atop complexes. The higher stability of the copper complex might be due to the two weakly held waters that could be displaced in the formation of the sitting-atop complex, allowing a more stable complex to exist.

Mechanism of Formation of Metalloporphyrins.— The kinetics of formation of metal tetrapyridylporphines in aqueous solutions can be formulated by the following scheme.

$$TPyPH_2 + 2H^+ \rightleftarrows TPyPH_4^{2+} \qquad K_D \qquad (6)$$

$$TPyPH_2 + M^{2+} \rightleftarrows M\text{-SAT-}TPyPH_2^{2+} \qquad K_{sat} \quad (7)$$

$$M\text{-SAT-}TPyPH_2^{2+} \rightleftarrows M\text{-SAT-}TPyPH^+ + H^+ \qquad K_{sat}' \qquad (8)$$

$$M\text{-SAT-}TPyPH^+ + M^{2+} \rightarrow M\text{-TPyP} + H^+ + M^{2+} \qquad k_1 \qquad (9)$$

If one assumes that steps 6, 7, and 8 are rapid pre-equilibrium steps, then an expression for k_{obsd} can be derived for this mechanism and it is given below.

$$k_{obsd} = \frac{k_1 K_{sat}' K_{sat} C_M^2}{(H^+)[1 + K_{sat}C_M + K_D(H^+)^2]} \qquad (10)$$

The experimental rate data fit this expression very well as illustrated in Fig. 4. Since we know K_{sat} (see Table I) and K_D (see ref. 4) we can obtain $k_1 K_{sat}'$ from the observed data.

Since we cannot determine K_{sat}', we always have the product of a rate constant and an equilibrium constant. This product $k_1 K_{sat}'$ is listed in Table II.

In most of the measurements the concentration of the $TPyPH_4^{2+}$ species was small enough to neglect (pH range 1.7–2.3 and $K_D = 5.4 \times 10^{-3}$; read ref. 4). There are two factors that slow down the rate of the reaction in acid solutions. One is the inverse hydrogen ion dependence and the other is the formation of the diacid species of the porphyrin. In 1.0 M acid the rate of the reaction is almost zero. This is because in 1.0 M acid the porphyrin is predominantly in the diacid species, $TPyPH_4^{2+}$. Since the diacid species is

known to be unreactive toward metals in metalloporphyrin formation, this again proves that the sitting-atop complex is not the diacid species.

As previously noted,[3] the reaction is inversely proportional to the hydrogen ion concentration for all the metals studied. We have postulated step 8, the loss of a proton from the porphyrin system, to account for this dependence.[17] The most unusual point of the mechanism is that the metal in the sitting-atop complex does not just drop into the porphyrin ring as previously postulated[15] but needs another metal ion to react with it to form the product metalloporphyrin. This may be due to the fact that the metal ion in the sitting-atop complex has its coordination waters trapped between the metal and the porphyrin through hydrogen bonding. This prevents the metal from entering the ring and another metal ion in solution that can lose its waters of coordination more easily reacts with the porphyrin to form the metalloporphyrin complex. Systems in which the sitting-atop complex cannot form, either because of the presence of strong coordinating ligands in the solution[3] or because of the porphyrin itself,[18] show a first-order dependence on the metal ion and therefore must react through a different path than the present system.

The order of the rates of the various metal ions is the following: Cu(II) > Zn(II) > Mn(II) > Co(II) > Fe(II) > Ni(II) > Cd(II). This order is essentially the same as obtained for other porphyrins.[3,19,20]

Experimental

The $\alpha,\beta,\gamma,\delta$-tetra(4-pyridyl)porphine and its metal complexes were prepared and purified as in ref. 2 and 3. All chemicals used were reagent grade and were recrystallized when necessary. The spectrophotometric measurements were taken on either a Cary Model 14 or Beckman DU spectrophotometer. The temperature was controlled in the cell compartment at 25.0°. The pH measurements were carried out on a Coleman pH meter that was calibrated before each set of measurements.

(16) H. Taube, H. Myers, and R. Rich, *J. Am. Chem. Soc.*, **75**, 4118 (1953).

(17) As pointed out by the editor the inverse hydrogen ion dependence can be explained just as well by an equilibrium $M^{2+} + H_2O \rightleftarrows MOH^+ + H^+$ followed by the reaction of the MOH^+ with $M\text{-SAT-}TPyPH_2^{2+}$. There is no way to distinguish between these alternatives from our kinetic data.

(18) E. Fleischer and P. Hambright, unpublished results.

(19) I. Shiller, K. Bernauer, and S. Fallab, *Experientia*, **17**, 540 (1961).

(20) R. A. Neve, "Haematin Enzymes," J. Falk, R. Lemberg, and R. Morton, Ed., Canberra, 1961.