phenomena by electrical means (*16*) dictates that considerable effort in these areas is more than justified, even if the present result in improving repair processes did not obtain.

The nature of the increased repair response deserves some comment. Although field-treated osteotomies generally were stronger than their contralateral unstimulated controls, the bulk of callus was less. This result stems most probably from the more highly organized nature of the "stimulated" callus, which may have served to stabilize the mobile fragments at an earlier time than the controls. Certainly, there was no evidence of increased cellularity or mitoses in the field-exposed fibulae. Furthermore, untraumatized soft tissues and bone exposed to the field appeared entirely normal.

Although, recently, this method has been applied successfully to treat a young girl with congenital pseudarthrosis of the tibia, its general use in fresh fractures remains to be defined. Should the present surgically noninvasive method prove to be effective in man, it may be possible to reduce fracture disability time significantly. In view of the relatively simple methodology, which would employ magnetic coils in plaster, such a reduction might well free hospital beds and alleviate a major economic drain.

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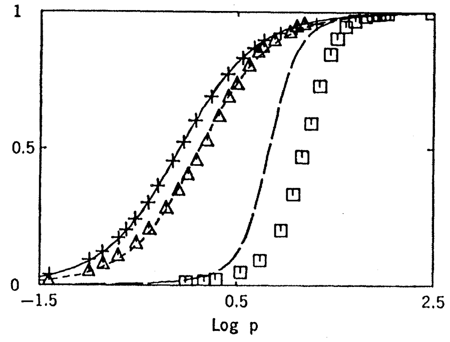
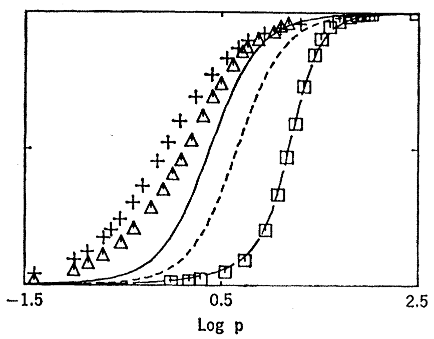
Oxygen Binding in Cyanmet Hybrid and Normal Hemoglobins: Applicability of Sequential and Two-State Concerted Models

Abstract. *The cyanmet hybrid hemoglobins* $\alpha_2\beta^{+\mathrm{CN}}_2$ *and* $\alpha^{+\mathrm{CN}}_2\beta_2$ *are widely held to be similar or equivalent in structure and subunit interactions to the partially oxygen-liganded species* $\alpha_2(\beta \cdot O_2)_2$ *and* $(\alpha \cdot O_2)_2\beta_2$*, respectively. An analysis of precise data on oxygen binding to the cyanmet hybrids and normal hemoglobin shows that if this is the case, then cooperative ligand binding in hemoglobin is more properly described by some model of the sequential type than by any two-state concerted model.*

For some years a controversy has existed over whether the cooperative ligand binding properties of hemoglobin may be more appropriately described by one of two classes of allosteric models. One class of models, called sequential, explicitly defines ligand-dependent pairwise-additive interactions between the four subunit chains (or hemes). According to this approach, the liganding of a subunit directly modifies the ligand affinity of other subunits with which it interacts. The first model of this type was proposed by Pauling (*1*) and the sequential concept has been subsequently broadened and extended by Coryell (*2*), Koshland *et al.* (*3*), Thompson (*4*), and Saroff (*5*). The other class of models, called concerted, treats the hemoglobin molecule as existing as an equilibrium mixture of distinct quaternary conformations, within each of which a subunit is defined as possessing an invariant ligand affinity. According to this approach, partial liganding of the hemoglobin shifts the equilibrium between quaternary conformations, thereby indirectly effecting an alteration in the (equilibrium average) oxygen affinity of remaining unliganded subunits. The concept of a concerted allosteric transition was first introduced by Monod, Wyman, and Changeux (*6*), who presented a formal two-state model for oligomers of identical subunits, referred to as the MWC model. Later this model was extended by Ogata and McConnell (*7*) to allow for possible nonequivalence of α and β chains in hemoglobin.

A number of investigators have gone to some lengths to show that a particular model is capable of rationalizing, or is compatible with, a variety of experimental data. However, in order to show that cooperative ligand binding in hemoglobin is more appropriately described by a sequential or concerted model, it is necessary to demonstrate that there exists some set of reliable experimental data which is compatible with only one of the two approaches. It is proposed that oxygen saturation data for the cyanmet hybrid hemoglobins $\alpha_2\beta^{+\mathrm{CN}}_2$ and $\alpha^{+\mathrm{CN}}_2\beta_2$, reported by Maeda *et al.* (*8*), together with oxygen saturation data for normal hemoglobin ($\alpha_2\beta_2$), reported by Tyuma *et al.* (*9*), constitute such a set. The data to be analyzed were obtained with the same apparatus under identical conditions for all three species, as specified in the legend to Fig. 1. These conditions are typical of those under which a large number of experiments on hemoglobin have been performed. Numerous experimental studies of the cyanmet hybrids have been undertaken with the objective of elucidating features of the mechanism of cooperative ligand binding in normal hemoglobin (*7, 10*). In all of these studies it has been either explicitly or implicitly assumed that the cyanmet hybrid $\alpha_2\beta^{+\mathrm{CN}}_2$ is similar if not identical in structure and subunit interactions to the partially oxygen-liganded species $\alpha_2(\beta \cdot O_2)_2$, and that the com-





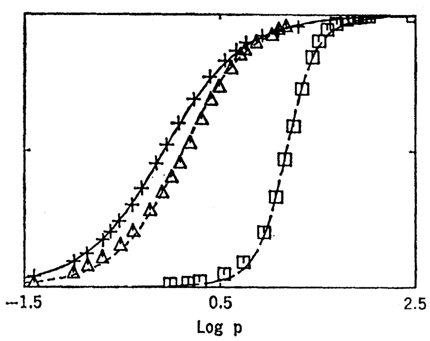


Fig. 1 (left). Oxygen saturation, *y*, versus the logarithm of the partial pressure of oxygen, *p* (torr). Data points are taken from (*8*) and (*9*); they were measured at 25°C with a heme concentration of (5 to 6) × $10^{-5}M$, in 0.05*M* 2,2-bis(hydroxymethyl)-2,2′,-2″-nitrilotriethanol, 0.1*M* NaCl, and 0.002*M* 2,3-diphosphoglycerate, at *p*H 7.4. The curves were calculated from the extended MWC model, as described in the text. (Crosses), $\alpha_2\beta^{+CN}{}_2$; (triangles), $\alpha^{+CN}{}_2\beta_2$; (squares) $\alpha_2\beta_2$. Fig. 2. (center). Plot of *y* versus log *p*. The curves were calculated from the extended MWC model, as described in the text. Data and symbols as in Fig. 1. Fig. 3 (right). Plot of *y* versus log *p*. The curves were calculated from the extended Coryell model, as described in the text. Data and symbols as in Fig. 1.

plementary hybrid $\alpha^{+CN}{}_2\beta_2$ is likewise equivalent in these respects to the partially oxygen-liganded species $(\alpha \cdot O_2)_2\beta_2$. It will be shown here that if this generally accepted view is correct, then (i) the extended MWC model is grossly incompatible with the data cited above (*8, 9*) and (ii) a simple sequential model containing the same number of variable parameters as the extended MWC model is fully compatible with the same data.

According to the extended MWC model, the oxygen equilibrium of hemoglobin is determined by five parameters which are independent of the partial pressure of oxygen when all other experimental conditions are held constant. These are the constant of conformational equilibrium between the unliganded R (oxy) and T (deoxy) conformations, designated L ($\equiv [\alpha_2\beta_2]_T/[\alpha_2\beta_2]_R$); the constants of oxygen association with α and β subunits in the R conformation, designated k_R^α and k_R^β, respectively; and the constants of oxygen association with α and β subunits in the T conformation, designated k_T^α and k_T^β, respectively.

The particular sequential model which we shall employ here is an extension of that proposed by Coryell (*2*) and quantified by Saroff and co-workers (*11, 12*). According to this model, the liganding of either of an interacting (unliganded) subunit pair results in the breaking of the ligand-dependent interaction between the two subunits. In the extended Coryell model, as in the extended MWC model, the oxygen equilibrium is determined by five parameters which are independent of the partial pressure of oxygen when all other experimental conditions are held constant. The intrinsic constant of oxygen association with a subunit, designated k_0, is assumed to be identical for α and β subunits. Modifications in affinity arising from interactions between the subunit pairs α_1-α_2, β_1-β_2, α_1-β_1 (or α_2-β_2), and α_1-β_2 (or α_2-β_1) are represented by multiplicative constants (interaction factors) respectively designated by α, β, γ, and δ.

The functional dependence of the oxygen saturation, *y*, on the partial pressure of oxygen, *p*, for normal hemoglobin and for the cyanmet hybrids may be expressed in the following general forms

$$y_{\alpha_2\beta_2}(p) = \frac{A_1p + 2A_2p^2 + 3A_3p^3 + 4A_4p^4}{4(A_0 + A_1p + A_2p^2 + A_3p^3 + A_4p^4)} \quad (1)$$

$$y_{\alpha_2\beta^{+CN}{}_2}(p) = \frac{B_1p + B_2p^2}{B_0 + 2B_1p + B_2p^2} \quad (2)$$

$$y_{\alpha^{+CN}{}_2\beta_2}(p) = \frac{C_1p + C_2p^2}{C_0 + 2C_1p + C_2p^2} \quad (3)$$

where the coefficients A_i, B_i, *and* C_i ($i = 1, 2, \ldots$) are model-dependent functions of the appropriate set of parameters.

According to the extended MWC model, the coefficients in Eqs. 1 to 3 are given by

$$\begin{aligned}
A_0 &= 1 + L \\
A_1 &= 2\,\{[k_R^\alpha + k_R^\beta] + L\,[k_T^\alpha + k_T^\beta]\} \\
A_2 &= [(k_R^\alpha)^2 + (k_R^\beta)^2 + 4k_R^\alpha k_R^\beta] + \\
&\qquad L\,[(k_T^\alpha)^2 + (k_T^\beta)^2 + 4k_T^\alpha k_T^\beta] \\
A_3 &= 2\,\{k_R^\alpha k_R^\beta\,(k_R^\alpha + k_R^\beta) + \\
&\qquad L\,[k_T^\alpha k_T^\beta\,(k_T^\alpha + k_T^\beta)]\} \\
A_4 &= [k_R^\alpha k_R^\beta]^2 + L\,[k_T^\alpha k_T^\beta]^2 \qquad (4a)
\end{aligned}$$

$$\begin{aligned}
B_0 &= 1 + L_\alpha \\
B_1 &= k_R^\alpha + L_\alpha k_T^\alpha \\
B_2 &= (k_R^\alpha)^2 + L_\alpha (k_T^\alpha)^2 \qquad (4b)
\end{aligned}$$

$$\begin{aligned}
C_0 &= 1 + L_\beta \\
C_1 &= k_R^\beta + L_\beta k_T^\beta \\
C_2 &= (k_R^\beta)^2 + L_\beta (k_T^\beta)^2 \qquad (4c)
\end{aligned}$$

where $L_\alpha \equiv]\alpha_2\beta^{+CN}{}_2]_T/[\alpha_2\beta^{+CN}{}_2]_R$ and $L_\beta \equiv [\alpha^{+CN}{}_2\beta_2]_T/[\alpha^{+CN}{}_2\beta_2]_R$.

If $\alpha_2\beta^{+CN}{}_2$ and $\alpha^{+CN}{}_2\beta_2$ are taken to be equivalent in structure and subunit interactions to the partially oxygen-liganded species $\alpha_2(\beta \cdot O_2)_2$ and $(\alpha \cdot O_2)_2\beta_2$, respectively—as is widely assumed—then it follows that

$$L_\alpha \approx \frac{[\alpha_2(\beta \cdot O_2)_2]_T}{[\alpha_2(\beta \cdot O_2)_2]_R} = L \left(\frac{k_T^\beta}{k_R^\beta}\right)^2$$

$$L_\beta \approx \frac{[(\alpha \cdot O_2)_2\beta_2]_T}{[(\alpha \cdot O_2)_2\beta_2]_R} = L \left(\frac{k_T^\alpha}{k_R^\alpha}\right)^2 \quad (5)$$

According to the extended Coryell model, the coefficients in Eqs. 1 to 3 are given by

$$\begin{aligned}
A_0 &= 1 \\
A_1 &= 2k_0\gamma\delta(\alpha + \beta) \\
A_2 &= k_0^2\gamma\delta(\alpha\gamma\delta + \beta\gamma\delta + 2\alpha\beta\delta + 2\alpha\beta\gamma) \\
A_3 &= 4k_0^3\alpha\beta\gamma^2\delta^2 \\
A_4 &= k_0^4\alpha\beta\gamma^2\delta^2 \qquad (6a)
\end{aligned}$$

$$\begin{aligned}
B_0 &= 1 \\
B_1 &= k_0\alpha \\
B_2 &= k_0^2\alpha \qquad (6b)
\end{aligned}$$

$$\begin{aligned}
C_0 &= 1 \\
C_1 &= k_0\beta \\
C_2 &= k_0^2\beta \qquad (6c)
\end{aligned}$$

The derivations of Eqs. 1 to 6 are omitted because they may be verified by readers familiar with the publication of Saroff and Yap (*12*). In addition, the reader may verify that, upon change of variables, Eq. 1 with coefficients given by Eqs. 4a is equivalent to equation 7 of (*7*).

The procedure described below was followed in examining the ability of the extended MWC model to accommodate the cited data (*8, 9*). First, Eq. 2 with coefficients given by Eqs. 4b (denoted for brevity by Eq. 2 + 4b) and Eq. 3 + 4c were simultaneously fitted to the data for $\alpha_2\beta^{+CN}{}_2$ and $\alpha^{+CN}{}_2\beta_2$, respectively, in order to determine best-fit values of the five parameters of the extended MWC model. The following values were obtained by fitting to the cyanmet hybrid data only: $k^{\alpha}_{T} = 0.0235$ torr^{-1}, $k^{\alpha}_{R} = 1.63$ torr^{-1}, $k^{\beta}_{T} = 0.0124$ torr^{-1}, $k^{\beta}_{R} = 1.25$ torr^{-1}, $L = 8169$. With these parameter values, the functional dependence of y on log p was calculated for $\alpha_2\beta_2$, $\alpha_2\beta^{+CN}{}_2$, and $\alpha^{+CN}{}_2\beta_2$ by using the extended MWC equations 1 + 4a, 2 + 4b, and 3 + 4c. The resulting curves are plotted together with the data in Fig. 1. It may be seen that, whereas the parameter values given above provide a satisfactory fit to the data for the two cyanmet hybrids (as expected), they do not provide a satisfactory fit to the data for normal hemoglobin.

An attempt was then made to find a set of parameter values which would simultaneously provide a satisfactory fit of Eqs. 1 + 4a, 2 + 4b, and 3 + 4c to the combined data for the cyanmet hybrids and normal hemoglobin. Although many initial combinations of parameter values were tried, the search was unsuccessful. As an example of the type of results obtained, curves of y versus log p are plotted in Fig. 2 for all three species; the curves are calculated from the following parameter values: k^{α}_{T} and k^{β}_{T} as above, $k^{\alpha}_{R} = k^{\beta}_{R} = 4.0$ torr^{-1}, $L = 9.52 \times 10^6$. It may be seen that these parameter values provide a satisfactory fit to the data for normal hemoglobin but do not provide even an approximate fit to the data for the cyanmet hybrids. It was found to be generally true that the better a set of parameter values fit the data for normal hemoglobin, the worse it fit the data for the cyanmet hybrids (and vice versa), irrespective of the particular parameter values selected. It may therefore be concluded that the extended MWC model is too restricted by the assumption expressed in Eqs. 5 to be capable of simultaneously accommodating the oxygen saturation data for both cyanmet hybrids and normal hemoglobin.

The procedure described below was followed in examining the ability of the extended Coryell model to accommodate the same data. First, Eqs. 2 + 6b and 3 + 6c were simultaneously fitted by least squares to the data for $\alpha_2\beta^{+CN}{}_2$ and $\alpha^{+CN}{}_2\beta_2$, respectively, in order to determine best-fit values of the parameters k_0, α, and β. The values of these three parameters were then fixed accordingly, and Eq. 1 + 6a was fitted to the data for $\alpha_2\beta_2$ to obtain best-fit values of γ and δ. The parameter values obtained in this manner are: $k_0 = 1.40$ torr^{-1}, $\alpha = 0.73$, $\beta = 0.30$, $[\gamma,\delta] = [0.58, 0.084]$ (*13*). With these parameter values, the functional dependence of y on log p was calculated for $\alpha_2\beta_2$, $\alpha_2\beta^{+CN}{}_2$, and $\alpha^{+CN}{}_2\beta_2$ by using the extended Coryell equations 1 + 6a, 2 + 6b, and 3 + 6c. The resulting curves are plotted together with the data in Fig. 3. It may be seen that, in contrast to the extended MWC model, the extended Coryell model is capable of simultaneously accommodating the oxygen saturation data for both cyanmet hybrids and normal hemoglobin.

The purpose of this report is not to demonstrate that the particular sequential model considered here (the extended Coryell model) provides an adequate description of ligand binding in hemoglobin. Indeed, there are several reasons for believing that it is highly oversimplified. Nonetheless, we have unequivocally demonstrated that if the widely accepted view that the cyanmet hybrid hemoglobins are similar or equivalent in structure and subunit interactions to their partially oxygen-liganded analogs is correct, then the cooperative ligand binding of hemoglobin is more properly described by some model of the sequential type than by any two-state concerted model.

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13. Because γ and δ enter symmetrically into Eq. 1 + 6a, it is not possible to determine which of the values in brackets corresponds to γ and which to δ without additional information.
14. I thank H. A. Saroff for helpful suggestions.

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Spontaneous Remembering after Recall Failure

Abstract. *Verbal free recall of lists of 20 items increased on repeated recall attempts, without any further presentation of each word after it had been recalled just once. Such restricted presentation resulted in long-term storage and retention of almost all 20 items, as shown by their eventual spontaneous retrieval without further presentation. Most items that failed to be recalled were retrieved again later without any further presentation, indicating that such failures represent retrieval failures rather than loss from storage and that free recall verbal learning requires retrieval from long-term storage.*

In order to show that recall failures during free recall verbal learning represent retrieval failures rather than loss of (information about) items from long-term storage (LTS) and to separate the effects of presentation on encoding and retrieval, the subjects in this study of spontaneous remembering were presented, on each trial, only those items not yet recalled at all, instead of all items in the list. Since such restricted presentation involves the presentation of each item only until it has been recalled once, while the subject attempts to recall all items in the list on each recall trial, the development of recall during such verbal learning can be examined without confounding due to continuing presentation. Each subject was tested individually; he was read a list of 20 items at a rate of 2 seconds per word and was asked for free recall of all items in the list in any order immediately after presentation. After this initial presentation of the entire list, the subject was presented only those items that had not yet been recalled at all, before he again attempted to recall all of the items in the list. Learning was