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WHY ARE THERE TWO KINDS OF CHAINS IN TETRAMERIC HEMOGLOBINS?*

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ABSTRACT

It is shown that the homeotropic allosteric interactions responsible for the sigmoidal oxygen saturation curves of $\alpha_2\beta_2$ hemoglobins are larger tha those of hypothetical hemoglobins obeying identical curves and built from equivalent chains, δ_4. It is also shown that this ensures for the $\alpha_2\beta_2$ species a more dependable cooperativety, through a biologically significative temperature range. On the basis of these findings it is argued that the existence of two different globin chains is advantageous in an evolutionary sense.

1. INTRODUCTION

From coelacanth to man, the hemoglobins of bony vertebrades are tetrameric proteins built from two kinds of polypeptide chains (Perutz. 1979). Human globin genes are arranged in two clusters, α and β, on two separate chromosomes. Reconstruction of the evolutionary path leads to the following picture (Maynard Smith, 1975; Jeffreys, 1982): $\sim 5 \times 10^8$ years ago the primitive jawless vertebrates (Agnatha) had a single-chain globin molecule in their blood (yhis also happens with the lampreys, which are survivous of this group). Sometime during the origin of the jawed vertebrades, $\sim 4.5 \times 10^8$ years ago, the globin-specifying gene duplicated and diverged, to become the genes coding for α and β chains. This paper is concerned with the question: it is possible to discern physico-chemical constrains which makes it biologically advantageous to have 0_2 carriers built from functionally non-equivalent binding sites?

We start from the assumption that the main biological role of hemoglobin is expressed by its sigmoidal oxygen saturation curve. This result from 0_2 molecules acting as homeotropic effectors, and from heterotropic effectors such as the hydrogen ion (the Bohr effect), $C0_2$, Ce^-, and organic phosphates (Monod, Wyman & Changeux, 1965). The latter operate a "fine tuning" of the cooperative behaviour, but since they are often variable or absent in the hempglobins of lower vertebrates they presumably evolved from a "core" of functional homeotropic effects (Antonini & Brunori, 1971, Eaton, 1980). It is with this homeotropic "core" that we are chiefly concerned in this paper.

2. GLOBINS WITH FUNCTIONALLY EQUIVALENT SITES

A single globin gene codes for a myoblobin-like monomeric protein which on oxygenation gives a hyperbolic curve:

$$\bar{Y} = \frac{k\, p_{O_2}}{1+k\, p_{O_2}} \qquad (1)$$

where $k = e^{-\Delta G^o/RT}$ is the oxygen binding constant. As shown in Figure 1 such a molecule is not an efficient dioxygen carrier between the respiratory interfaces ($p_{O_2} \approx 100$ torr) and muscle and other tissues ($p_{O_2} \approx 10$-20 torr)*. In vertebrates this role is played by hemoglobin.

Since the carefull work of Gilbert Adair (1925) hemoglobin is known to contain four hemes per molecule, each heme binded to one globin chain. Assuming that the four hemes are functionally equivalent, the fractional saturation can be described (Adair, 1925; Baldwin , 1975) in terms of four intrinsic (i.e., corrected for statistical factors) oxygen binding constants:

$$\bar{Y} = \frac{k_1 p_{O_2} + 3k_1k_2 p_{O_2}^2 + 3k_1k_2k_3 p_{O_2}^3 + k_1k_2k_3k_4 p_{O_2}^4}{1 + 4k_1 p_{O_2} + 6k_1k_2 p_{O_2}^2 + 4k_1k_2k_3 p_{O_2}^3 + k_1k_2k_3k_4 p_{O_2}^4} \qquad (2)$$

If there is no interaction between liganded and non-liganded sites, i.e., if $k_1=k_2=k_3=k_4$, curve (2) is hyperbolic. However, if $k_3k_4 > k_1k_2$ that is, if there are appropriate allosteric interactions between liganded and non-liganded subunits,

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* The O_2 content of the Earth athmosphere has remained sensibly constant since 2.5×10^9 years ago, i.e., long before the vertebrates first appeared.

equation (2) gives a sigmoidal curve (Pauling, 1935; Pauling & Coryell, (1936); Coryell (1939)) as required from an efficient dioxygen carrier.

Careful hemoglobin oxygenation data are now available, and it is possible in many cases to calculate the individual allosteric free-energies, ($G_i = -RT\ \ell n g_i$), where $g_i = \frac{k_{i+1}}{k_i}$. Some of the more recent values for human hemoglobins are shown in Table I, and sigmoidal curves are depicted in Figure 2 (Tyuma, Imai & Shimizu, 1973).

Since these curves can be described in terms of four equivalent subunits, the existence of two distinct globin genes could be seen as an unecessary complication, probably the result of neutral mutations. We are struck, however, by the small values of the sequential allosteric free-energies, calculated for hemoglobin molecules with equivalent sites. On the basis of the current knowledge of the role played by subunit interfaces in the allosteric interactions (Shulman, et. al, 1969; Perutz, 1970, 1972; Chotia, Wodik & Janin, 1976) we will argue that the safe performance of cooperative binding through a biologically meaningful temperature range demands G_i^o values substantially larger than those shown on Table I.

Let us consider first the oxygenation of a hypothetical δ_2 dimeric protein. Equation (2) reduces to

$$\bar{Y} = \frac{k_1 p_{O_2} + k_1 k_2 p_{O_2}^2}{1 + 2k_1 p_{O_2} + k_1 k_2 p_{O_2}^2} \qquad (3)$$

$\bar{Y}$ is hyperbolic if $k_1 = k_2$ (Figure 3, curve (a)), or if $k_1 < k_2$

(Figure 3, curve (b)). In the first case, $g_1 = \frac{k_2}{k_1} = 1$ (non-cooperativity; $G_1^o = 0$), in the latter, $g_1 = \frac{k_2}{k_1} < 1$ (allosteric anticooperativity; $G_1^o > 0$).

Cooperativity, resulting in a sigmoidal curve, is obtained if $k_2 = g_1 k_1 > k_1$ ($G_1^o < 0$; Figure 3, curve (c)). How could one garantee that the condition $g_1 > 1$ is maintained over a significant temperature range? The temperature dependence of equilibrium constants is given, to a good approximation, by the expression:

$$\ln k_i(T) = - \frac{\Delta H_i(0)}{RT} + \frac{\Delta a_i}{R} \ln T + \frac{\Delta b_i}{2R} T + \ldots \qquad (4)$$

For the two successive oxygenations of the dimer:

$$\begin{aligned} &\delta_2 + O_2 \rightleftharpoons \delta_2(O_2) \; ; \; k_1 \\ &\delta_2(O_2) + O_2 \rightleftharpoons \delta_2(O_2)_2 \; ; \; k_2 \end{aligned} \qquad (5)$$

one obtains:

$$\ell n \frac{k_2}{k_1} = - \left[\frac{\Delta H_2(0) - \Delta H_1(0)}{RT}\right] + \frac{\Delta a_2 - \Delta a_1}{R} \ell n \, T + \frac{\Delta b_2 - \Delta b_1}{2R} T + \ldots \qquad (6)$$

The allosteric enthalphy at 0^oK is the term $H_1^o = \Delta H_2(0) - \Delta H_1(0)$; only if it is large and negative, one could be sure that the ratio $\frac{k_2}{k_1}$ stays larger than 1, even if the heat capacity terms, in a given temperature range, are unfavorable. This is the "safety requirement" for a diatomic protein. As shown below, in tetrametric proteins cooperativity occurs if $k_1 k_2 < k_3 k_4$; but the "safety requirement" remains valid in that the allosteric energies should be large enough not to be "overshooted" by unfavorable heat capacity factors.

This "safety margin" is important on two counts: a) most vertebrates, certainly the primitive ones, are poikilo - thermous, and their hemoglobin should be dependable in the temperature range 0 to 50°C.

b) The recent realization that a protein with N aminoacids has $\sim e^N$ closed spaced states, making it a very flexible entity (Janin et. al., 1978; Gelin & Karplus, 1979; Fraunfelder et. al., 1980; Artymink, et.al.1980) predicts large variations in the heat--capacities with the induced-fit changes accompaning hemoglobin oxygenation (Koshland, Nemethy & Filmer, 1966; Koshland & Neet, 1968; Perutz, 1970, 1979).

We have analysed the behaviour of mock heme-contaning proteins built from four equivalent subunits with large allosteric interactions. Figure 4 shows the results of some of our attempts. It seems that large allosteric interactions would not make δ_4 iron-proteins efficient dioxygen carriers at the physiological range of p_{O_2} values. We conclude that for n functionally equivalent subunits, the appropriate allosteric tuning must remain dangerously close to the environmental k_BT values.

3. DIMERS WITH NON-EQUIVALENT BINDING SITES

We know for a fact that all vertebrate hemoglobins are built from two pairs of different globin chains. For example, in normal human adult hemoglobin the α chains have a different (probably larger) O_2 affinity from that of the β chains. This introduces a new factor in our discussion, which can be summarized as follows: non-interacting equivalent binding sites

leads to simple non-cooperativity ($k_1=k_2=...=k_n$). On the other hand, non-interaction between <u>different</u> binding sites leads to non-allosteric anticooperativity, i.e., $k_{i+1} < k_i$ (Mills & Ackers, 1979). As a result of this effect, to achieve a given level of cooperativity it is necessary in the latter case, to introduce <u>larger</u> allosteric energies.

For simplicity, consider first the case of a dimeric protein, αβ. Its dioxygen equilibria is shown in the diagram of Figure 5. The fractional saturation is:

$$\bar{Y} = \frac{K_1 p_{O_2} + 2K_1K_2p_{O_2}^2}{2\left[1+K_1p_{O_2} + K_1\,K_2p_{O_2}^2\right]} \qquad (7)$$

Where K_1 and K_2 are the extrinsic binding constants, given by $K_1 = k_\alpha + k_\beta$ and $K_2 = \frac{k_\alpha k_{\alpha\beta}}{k_\alpha+k_\beta}$.

Although in this case there is no difference between intrinsic and extrinsic binding constants, the $\bar{Y}$ curve will be sigmoidal (cooperative binding) according to the same criterion valid for eq. (3), namely, if $k_1 = \frac{1}{2} K_1 < k_2 = 2K_2$; otherwise the curve is hyperbolic.

If $k_\alpha >> k_\beta$, then $k_1 \to k_\alpha$ and $k_2 \to k_{\alpha\beta}$; if, furthermore, there is no allosteric interaction ($k_{\alpha\beta} \to k_\beta$), it follows that $k_1 >> k_2$. The binding will be <u>anticooperative</u>, the first O_2 molecule binding on the average tighter than the second one. This effect does not occur if $k_\alpha = k_\beta$ (and $k_{\alpha\beta} = k_\beta$). Thus:

$$k_1 = \frac{K_1}{2} = \frac{k_\alpha + k_\beta}{2} = \frac{2k_\alpha}{2} = k_\alpha \qquad (8)$$

and

$$k_2 = 2K_2 = \frac{2k_\alpha k_{\alpha\beta}}{k_\alpha + k_\beta} = \frac{2k_\alpha^2}{2k_\alpha} = k_\alpha \qquad (9)$$

Therefore, $k_1 = k_2$, and the process is just non-cooperative.

How large can be this anticooperative effect? Suppose we have non-interacting subunits, and that $k_\alpha >> k_\beta$. Clearly:

$$\lim_{k_\beta \to 0} k_1 = k_\alpha \qquad (10)$$

and

$$\lim_{k_\beta \to 0} k_2 = \lim_{K_\beta \to 0} \frac{k_\alpha k_{\alpha\beta}}{k_\alpha + k_\beta} = 0 \qquad (11)$$

This gives $\lim_{k_\beta \to 0} (\Delta G_1^o - \Delta G_2^o) = \infty$, which shows that the effect can be very large.

The sigmoidal condition for equation (7), $k_1 < k_2$, is equivalent to the inequality:

$$e^{-\frac{\Delta G_\alpha^o - \Delta G_\beta^o}{RT}} + e^{-\frac{\Delta G_\beta^o - \Delta G_\alpha^o}{RT}} + 2 < 4\, e^{-\frac{G_\alpha^o}{RT}} \qquad (12)$$

where $G_\alpha^o = \Delta G_{\alpha\beta}^o - \Delta G_\beta^o$. As $|\Delta G_\alpha^o - \Delta G_\beta^o|$ increases, the sigmoidal shape is maintained only if the allosteric term G_α^o also increases.

The effect is shown in graphic form in Figure 6. Curve (a) is the one for the αβ dimer of human hemoglobin A according to Mills & Ackers (1979). Within the experimental errors, there is no allosteric interaction in the dimer, the curve being slightly anticooperative ($|\Delta G_\alpha^o - G_\beta^o| = 280$ cal. $mole^{-1}$).

For comparison, curve (b) of Fig. 6 was drawn for a δ_2 dimer with equivalent sites ($\Delta G^o_\delta = -8.070$ cal. mole^{-1}); the curve is strictly non-cooperative. On the other hand curve (c) is very anti-cooperative, having been calculated for an $\alpha\beta$ dimer in which $\Delta G^o_\alpha = -9{,}070$ cal. mole^{-1} and $\Delta G^o_\beta = -6{,}000$ cal. mole^{-1}, and without allosteric interaction ($\Delta G^o_{\alpha\beta} = \Delta G^o_\beta$).

In curve 6 (d), $\Delta G^o_\alpha = -6{,}000$ cal. mole^{-1}, and $\Delta G^o_\beta = -4{,}000$ cal. mole^{-1}, but we introduce an allosteric free-energy of -4,000 cal. mole. Finally, in curve 6 (e) the allosteric interaction is the same but $|\Delta G^o_\alpha - \Delta G^o_\beta|$ is 3,000 cal. mole^{-1}. The sigmoidal shapes of curves (d) and (e) of Fig. 6 reflect the importance of the magnitude of $|\Delta G^o_\alpha - \Delta G^o_\beta|$ upon cooperativity.

4. TETRAMERIC HEMOGLOBINS

In the case of tetrameric hemoglobins with two different pairs of chains, $\alpha_2\beta_2$, incorporation of the chain heterogenity into the Adair scheme increases enormously the number of undetermined constants (Tyuma, Imai & Shimizu, 1973). The full scheme is shown in Figure 7; the fractional saturation $\bar{Y}$ is given by:

$$\bar{Y} = \frac{K_1 p_{O_2} + 2K_1K_2p_{O_2}^2 + 3K_1K_2K_3p_{O_2}^3 + 4K_1K_2K_3K_4p_{O_2}^4}{4\left[1 + K_1p_{O_2} + K_1K_2p_{O_2}^2 + K_1K_2K_3p_{O_2}^3 + K_1K_2K_3K_4p_{O_2}^4\right]} \qquad (13)$$

where:

$$K_1 = 2k_\alpha + 2k_\beta \qquad (14)$$

$$K_2 = \frac{k_\alpha k_{\alpha\alpha} + 2k_\alpha k_{\alpha_I\beta_I} + 2k_\alpha k_{\alpha_I\beta_{II}} + k_\beta k_{\beta\beta}}{2k_\alpha + 2k_\beta} \quad (15)$$

$$K_3 = \frac{2k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} + 2k_\alpha k_{\alpha_I\beta_I} k_{\beta_I\alpha_I\beta_{II}}}{k_\alpha k_{\alpha\alpha} + 2k_\alpha k_{\alpha_I\beta_I} + k_\beta k_{\beta\beta} + 2k_\alpha k_{\alpha_I\beta_{II}}} \quad (16)$$

$$K_4 = \frac{k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} k_{\alpha\alpha\beta\beta}}{2k_\alpha k_{\alpha\alpha} k_{\alpha\alpha\beta} + 2k_\alpha k_{\alpha_I\beta_I} k_{\beta_I\alpha_I\beta_{II}}} \quad (17)$$

According to Figure 7, there are 16 equilibrium constants involved, but there are also 7 free-energy conservation constrains, which allows one to retain only 9 constants; for example, k_α, k_β, $k_{\alpha\alpha}$, $k_{\beta\beta}$, $k_{\alpha_I\beta_I}$, $k_{\alpha_I\beta_{II}}$, $k_{\beta_I\alpha_I\beta_{II}}$, $k_{\alpha\alpha\beta}$, and $k_{\alpha\alpha\beta\beta}$ (this is one possible set). We make the following "strong" approximations:

1. $k_\alpha = k_{\alpha\alpha}$; this is based in the fact that the number and type of salt-bridges broken in the oxygenation of one α subunit is independent of the occupancy of the other α subunit (Perutz, 1970, 1972).

2. $k_\beta = k_{\beta\beta} = k_{\alpha_I\beta_I}$; from the work of Perutz (1970, 1979); Baldwin, (1975) we know that the ββ and $\alpha_I\beta_I$ subunit interfaces are not modified by oxygenation. We know furthermore that both the β_4 tetramer (Benesch & Benesch, 1961; Baldwin, 1975), and the αβ dimer (Mills & Ackers, 1979; Hewitt, 1979) are non-cooperative within the experimental errors.

Under these approximations the 9 constants are reduced to 6; for example, k_α, k_β, $k_{\alpha_I\beta_{II}}$, $k_{\alpha\alpha\beta}$, $k_{\beta_I\alpha_I\beta_{II}}$, and $k_{\alpha\alpha\beta\beta}$. We eliminate two further constants by assuming a "less

strong" approximation, viz., that the dominant allosteric interaction, $\Delta G^{o}_{\alpha_I\beta_{II}}$, is independent of the oxygen occupancy of the other α site (hence, $k_{\alpha\alpha\beta} = k_{\alpha_I\beta_{II}} = k'_{\alpha\beta}$), but dependent of the occupancy of the second β site (hence, $k_{\beta_I\alpha_I\beta_{II}} = k_{\alpha\alpha\beta\beta} = k''_{\alpha\beta}$). This is strictly true for effectors such that one single molecule binds to one site in the ββ interface, v.g., diphosphoglycerate.

We end-up with four constants, k_α, k_β, $k'_{\alpha\beta}$ and $k''_{\alpha\beta}$, and we can solve the system of equation (14)-(17). Applying this analysis to the same data used to construct Table I (Tyuma, Imai & Schimizu (1973)) we obtained the results shown in Tables II and III. The two last columns in each Table show the allosteric free-energies, and these should be compared with the G^{o}_{i} values of Table I.

The first point to be stressed is that cooperativity in the oxygenation of an $\alpha_2\beta_2$ tetrameric protein is not as clear-cut as in a dimer (for example, one may have $k_2 < k_1$, and the curve may still be sigmoidal).

An analysis shows (Jacchieri, 1982) that cooperativity requires that

$$k_1 k_2 < k_3 k_4 \qquad (18)$$

where the small ks refer to the intrinsic dioxygen binding constants, $k_1 = \frac{1}{4} K_1$, $k_2 = \frac{2}{3} K_2$, $k_3 = \frac{3}{2} K_3$ and $k_4 = 4K_4$. In terms of the binding free-energies, ΔG^{o}_{α}, ΔG^{o}_{β}, $\Delta G^{o}_{\alpha_I\beta_{II}}$, etc., condition (18) corresponds to:

$$e^{-\frac{\Delta G^{o}_{\alpha} - \Delta G^{o}_{\beta}}{RT}} < 6e^{-\frac{\Delta G^{o}_{\alpha\beta\beta} - \Delta G^{o}_{\beta} + \Delta G^{o}_{\alpha\alpha\beta\beta} - \Delta G^{o}_{\beta}}{2RT}} - 2e^{-\frac{\Delta G^{o}_{\alpha_I\beta_{II}} - \Delta G^{o}_{\beta}}{RT}} \qquad (19)$$

The same general considerations we made while discussing the α β dimer can be replaced starting with the system of equations (14)-(17). Thus, if there are no allosteric interactions ($k_{\alpha_I\beta_I} = k_{\alpha_I\beta_{II}} = k_{\beta\beta} = k_{\beta}$), and if $|\Delta G^{o}_{\alpha}| >> |\Delta G^{o}_{\beta}|$, we will find that

$$k_i = k_{\alpha} = \frac{K_1}{2} \qquad (20)$$

$$k_2 = k_{\alpha} = 2\,K_2 \qquad (21)$$

$$k_3 = k_4 = 0 \qquad (22)$$

Hence, the first two dioxygen molecules will bind much tighter than the two last ones, giving rise to non-allosteric anti-cooperativity. It is seen from inequality (19) that if $|\Delta G^{o}_{\alpha} - \Delta G^{o}_{\beta}|$ is large, binding in hemoglobin will be anti-cooperative unless the magnitude of the terms $\Delta G^{o}_{\alpha\alpha\beta\beta}$ and $\Delta G^{o}_{\alpha\beta\beta}$ are much larger than that of ΔG^{o}_{β}. But, of course, this means that the allosteric interactions across the $\alpha_I\beta_{II}$ interfaces, $\Delta G^{o}_{\alpha\beta\beta} - \Delta G^{o}_{\beta}$ and $\Delta G^{o}_{\alpha\alpha\beta\beta} - \Delta G^{o}_{\beta}$ must be large (and negative).

These results are born by the last columns of Tables II and III. They can be seen as a direct consequence of attributing most of the allostery shown in the oxygenation of hemoglobin to changes in the $\alpha_I\beta_{II}$ and $\alpha_{II}\beta_I$ interfaces, as required by Perutz model (Perutz, 1970).

The decrease in the value of the oxygen affinity of hemoglobin caused by the addition of DPG and other organic phosphates is well known (Benesch & Benesch, 1974). This is rationalized in the two-state model of the hemoglobin - O_2 equilibrium (Monod, Wyman & Changeux, 1965) by supposing that the DPG molecule binds more strongly with the deoxy-form (T) than with the oxy-form (R). Perutz has shown (1970) that DPG binds across the ββ interface of deoxyhemoglobin. The results shown in Tables II and III indicate that as one increases the concentration of DPG, $|\Delta G_\beta^o|$ decreases faster than $|\Delta G_\alpha^o|$; the calculated allosteric interactions therefore increases. The MWC model predicts the observed increase of the P_{50} of hemoglobin in the presence of DPG but hardly the change in the shape of the saturation curve. The inclusion of site heterogenity, however, explains the latter effect. As the evidence of a preferential sequence in the oxygenation of hemoglobin ($\alpha O_2\alpha\beta\beta \rightarrow \alpha O_2\alpha O_2\beta\beta \rightarrow \alpha O_2\alpha O_2\beta O_2\beta \rightarrow \alpha O_2\alpha O_2\beta O_2\beta O_2$) becomes more quantitative (Lindström & Ho, 1972; Johnson & Ho, 1974) it will be possible to compare the results in the light of the non-equivalent site model, and to understand better the heterotropic allosteric interactions.

5. Conclusions

It is generally agreed that the point mutation responsible for the diversity of aminoacid composition and/or sequency in hemoglobins are mostty neutral. We have argued in this paper that definite physicochemical constrains made the primordial globin gene duplication and functional divergence advantageous. We have, in fact, raised a question and proposed an answer to a problem of biological specificity at a level deeper than hitherto thought possible. In a Popperian seuse, such questions in the theory of evolution do not have the "strong" scientific requirement of being disprovable. It must be judged rather in terms of plausibility. We believe that that our proposal meets this test quite well.

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TABLE I

ALLOSTERIC FREE-ENERGIES IN HUMAN HEMOGLOBINS

Hemoglobin	G_1^o (kcal.mole^{-1})	G_2^o (Kcal.mole^{-1})	G_3^o (Kcal.mole^{-1})
Stripped Human A	-0.22±0.17	-1.14±0.28	-0.72±0.19
Human A (2 mM DPG)	+0.24±0.18	-0.92±0.36	-2.67±0.28
Stripped Human F	-0.66±0.17	-0.16±0.24	-1.63±0.22
Human F (2 mM DPG)	+0.14±0.28	-0.90±0.66	-2.36±0.65
Human A (1.7 mM IHP)	-0.34±0.12	+0.46±0.26	-2.79±0.35

All temperatures are 298°K (k_BT = 0.6 kcal. mole^{-1})

Conversion factor: $[0_2(aq)]$ (mole.ℓ^{-1}) = 1.23 x 10^{-6} p_{0_2} (torr)

TABLE II

HUMAN HEMOGLOBINS IN THE ABSENCE OF NaCℓ

Hemoglobin	[DPG] (mM)	ΔG^{o}_{α} (kcal.mole^{-1})	ΔG^{o}_{β} (kcal.mole^{-1})	$\Delta G^{o'}_{\alpha\beta}$ kcal.mole^{-1})	$\Delta G^{o''}_{\alpha\beta}$ (kcal.mole^{-1})	$\Delta G^{o}_{\alpha} - \Delta G^{o}_{\beta}$ (kcal.mole^{-1})	$G^{o'}_{\alpha\beta}$ (kcal.mole^{-1})	$G^{o''}_{\alpha\beta}$ (kcal.mole^{-1})
Human F	0.0	-6.88±0.10	-5.15±0.40	-7.29±0.16	-8.88±0.24	-1.73±0.42	-2.14±0.43	-3.73±0.47
Human F	2.0	-6.27±0.10	-2.48±0.90	-4.86±0.85	-9.78±0.87	-3.79±0.91	-2.38±1.50	-7.30±1.50
Human A	0.0	-7.13±0.10	-5.47±0.35	-6.90±0.25	-9.58±0.30	-1.67±0.37	-1.42±0.44	-4.11±0.47
Human A	2.0	-5.90±0.10	-2.08±0.71	-4.76±0.66	-9.56±0.68	-3.82±0.72	-2.68±0.97	-7.48±0.99

The allosteric free-energies are in the last two columns:

$G^{o'}_{\alpha\beta} = \Delta G^{o'}_{\alpha\beta} - \Delta G^{o}_{\beta}$, and $G^{o''}_{\alpha\beta} = \Delta G^{o''}_{\alpha\beta} - \Delta G^{o}_{\beta}$

TABLE III

ADULT HUMAN HEMOGLOBIN IN NaCℓ 0.1M

[DPG]	ΔG_{α}^{o}	ΔG_{β}^{o}	$\Delta G_{\alpha\beta}^{o'}$	$\Delta G_{\alpha\beta}^{o''}$	$\Delta G_{\alpha}^{o} - \Delta G_{\beta}^{o}$	$G_{\alpha\beta}^{o'}$	$G_{\alpha\beta}^{o''}$
(mM)	(kcal. $mole^{-1}$)	(kcal.$mole^{-1}$)	(kcal.$mole^{-1}$)	(kcal.$mole^{-1}$)	(kcal.$mole^{-1}$)	(kcal.$mole^{-1}$)	(kcal.$mole^{-1}$)
0.0	-6.24±0.10	-3.41±0.54	-6.60±0.17	-9.10±0.28	-2.83±0,55	-3.19±0.57	-5.65±0.61
0.2	-6.13±0.10	-2.17±0.55	-5.38±0.42	-9.52±0.45	-3.97±0.56	-3.22±0.70	-7.35±0.72
0.5	-5.90±0.11	-0.99±2.00	-5.93±0.35	-8.96±0.47	-4.91±2.00	-4.94±2.04	-7.96±2.06
1.0	-5.88±0.10	-1.65±0.92	-4.77±0.85	-9.95±0.88	-4.25±0.93	-3.14±1.26	-8,33±1.28

The allosteric free-energies are in the last two columns:

$G_{\alpha\beta}^{o'} = \Delta G_{\alpha\beta}^{o'} - \Delta G_{\beta}^{o}$, and $G_{\alpha\beta}^{o''} = \Delta G_{\alpha\beta}^{o''} - \Delta G_{\beta}^{o}$

FIGURE CAPTIONS

FIGURE 1 - Oxygen saturation curve of myoglobin. A tetrameric protein with four independent equivalent sites would give the same curve.

FIGURE 2 - Oxygen saturation curves of hemoglobins.

——— Hb-A, 2mM DPG, [NaCℓ] = 0.0

---- Hb-A, stripped

■■■■ Hb-F, 2mM DPG, [NaCℓ] = 0.0

-·-·- Hb-A, Mills & Ackers (1979)

FIGURE 3 - Oxygen saturation curves for a δ_2 dimer

Curve (a) ——— Non-cooperative behaviour
($\Delta G^{o}_{\delta} = \Delta G^{o}_{\delta\delta} = -7.000$ cal. mole^{-1})

Curve (b) ----- Anti-cooperative behaviour
($\Delta G^{o}_{\delta} = -7.000$ cal.mole^{-1};
$\Delta G^{o}_{\delta\delta} = -6.000$ cal.mole^{-1})

Curve (c) -·-·- Cooperative behaviour
($\Delta G^{o}_{\delta} = -6.000$ cal.mole^{-1};
$\Delta G^{o}_{\delta\delta} = -8.000$ cal.mole^{-1})

FIGURE 4 - Oxygen saturation curves of δ_4 tetramer with large allosteric free-energies

——— Hb-A, 2.0mM DPG, [NaCℓ] = 0.1; curve shown for comparative purpose

----- Same Hb-A; starting with same k_4 value, but with $G_4^o = G_3^o = G_2^o = -2010$ cal.mole^{-1}

-·-·- Same Hb-A; starting with same k_1 value, but with $G_2^o = G_3^o = G_4^o = -1770$ cal.mole^{-1}

FIGURE 5 - $\alpha\beta - O_2$ equilibria

FIGURE 6 - Curve (a) ——— Experimental curve for the αβ dimer at 37° (Mills & Ackers, 1979)
$\Delta G_\alpha^o = -8.070$ cal.mole^{-1};
$\Delta G_\beta^o = -7.790$ cal. mole^{-1}

Curve (b) ----- δ_2 dimer, non-cooperative
($\Delta G_\delta^o = \Delta G_{\delta\delta}^o = -8.070$ cal.mole^{-1})

Curve (c) ••••• αβ dimer, non-allosteric anti-cooperative ($\Delta G_\alpha^o = -8.070$ cal.mole^{-1};
$\Delta G_\beta^o = -6.000$ cal.mole^{-1})

Curve (d) ▲▲▲▲▲ αβ dimer, cooperative
($\Delta G_\alpha^o = -6.000$ cal.mole^{-1};
$\Delta G_\beta^o = -4.000$ cal.mole^{-1}
$\Delta G_{\alpha\beta}^o = -8.000$ cal.mole^{-1})
Allosteric free-energy: -4.000 cal.mole^{-1}

Curve (e) ■■■■■ αβ dimer, cooperative
($\Delta G_\alpha^o = -6.000$ cal.mole^{-1};
$\Delta G_\beta^o = -3.000$ cal.mole^{-1}
$\Delta G_{\alpha\beta}^o = -7.000$ cal.mole^{-1})
Allosteric free-energy: -4.000 cal.mole^{-1}

FIGURE 7 - The complex equilibria $Hb\text{-}O_2$ including site heterogenity.

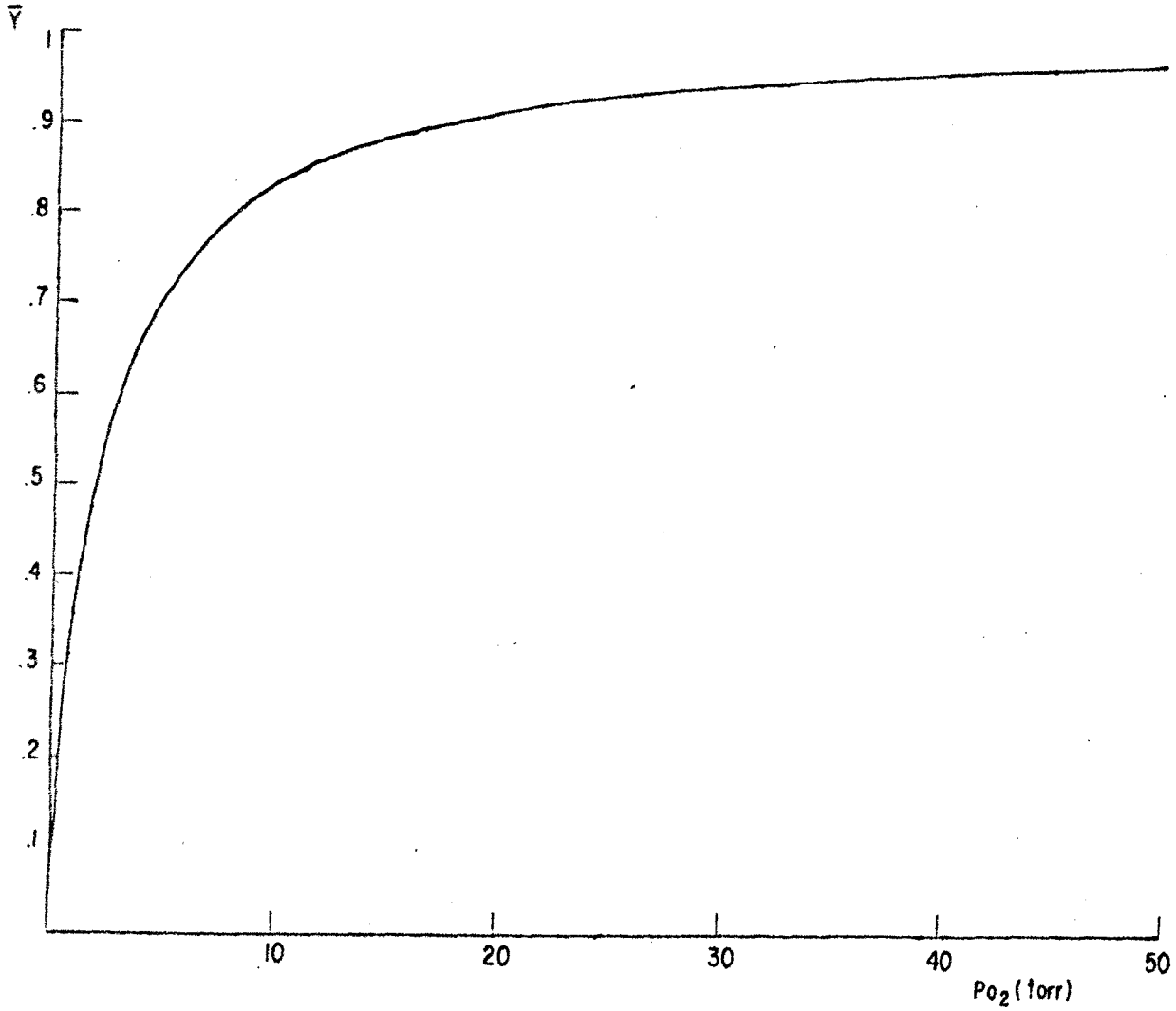

FIGURE 1

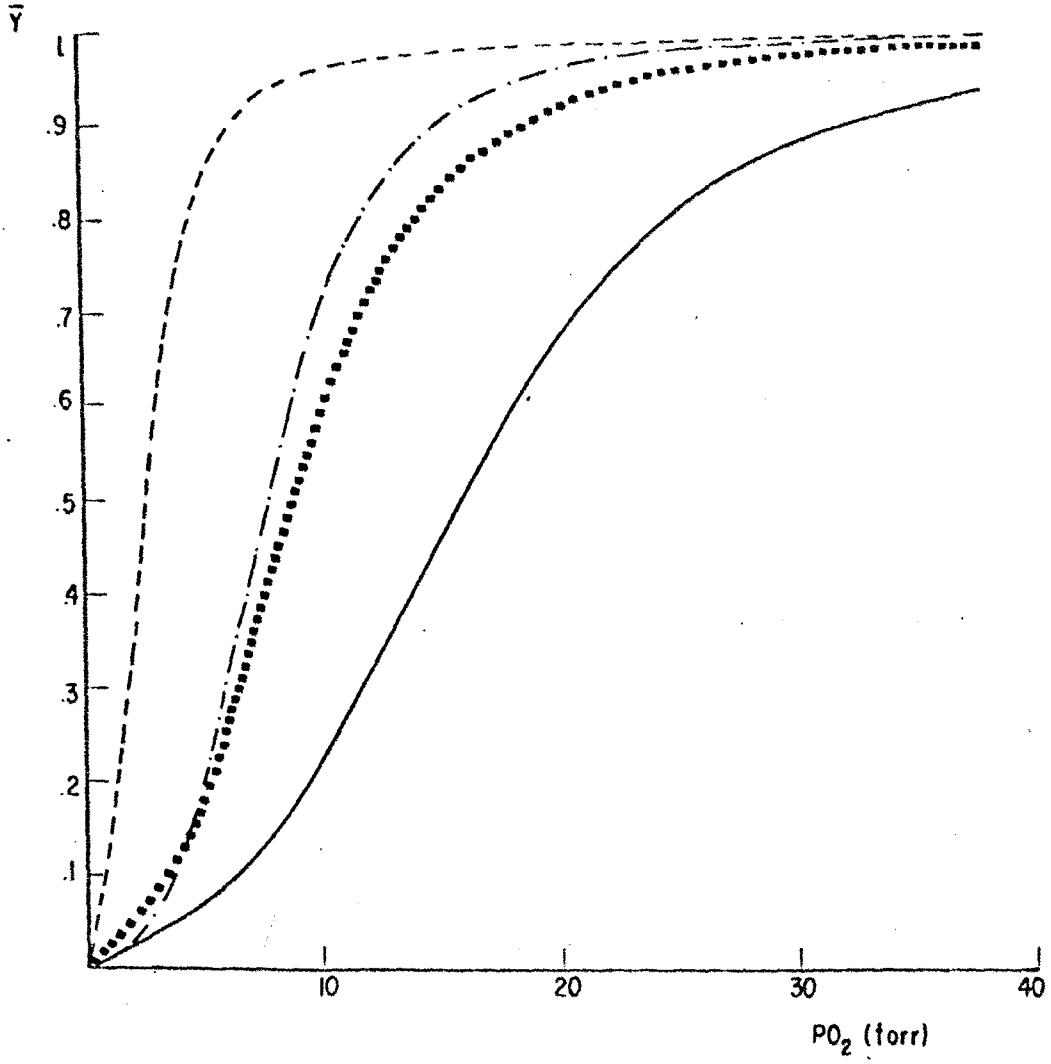

FIGURE 2

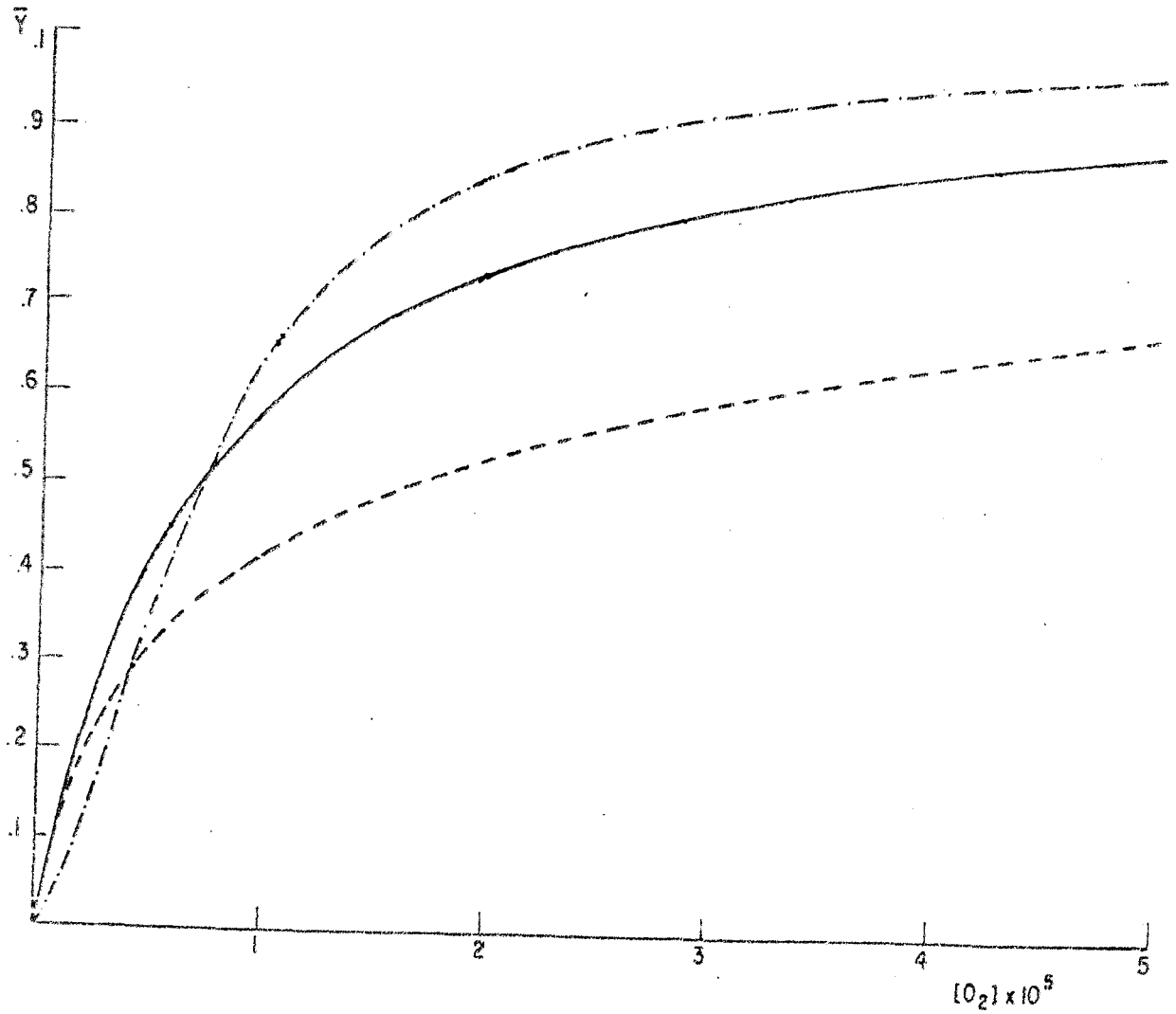

FIGURE 3

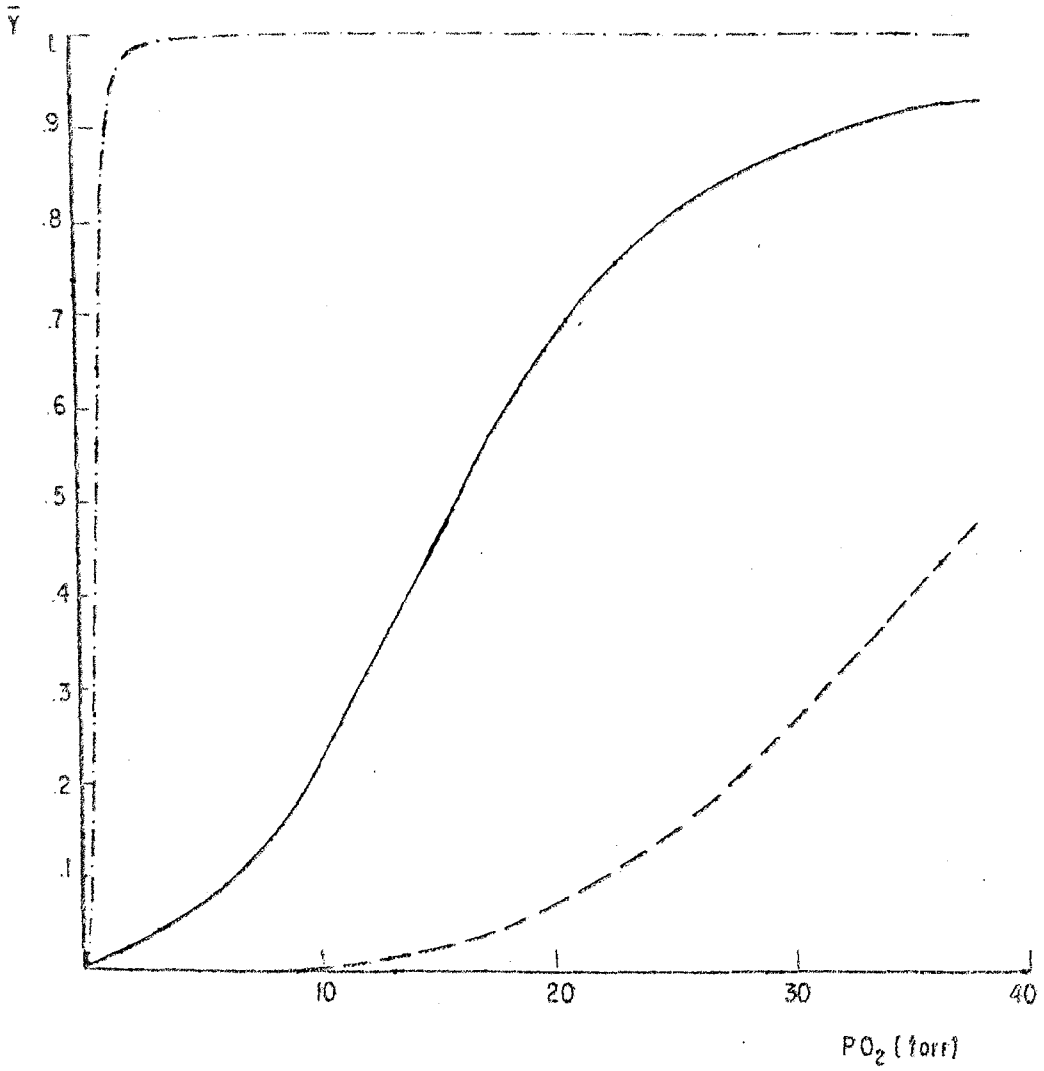

FIGURE 4

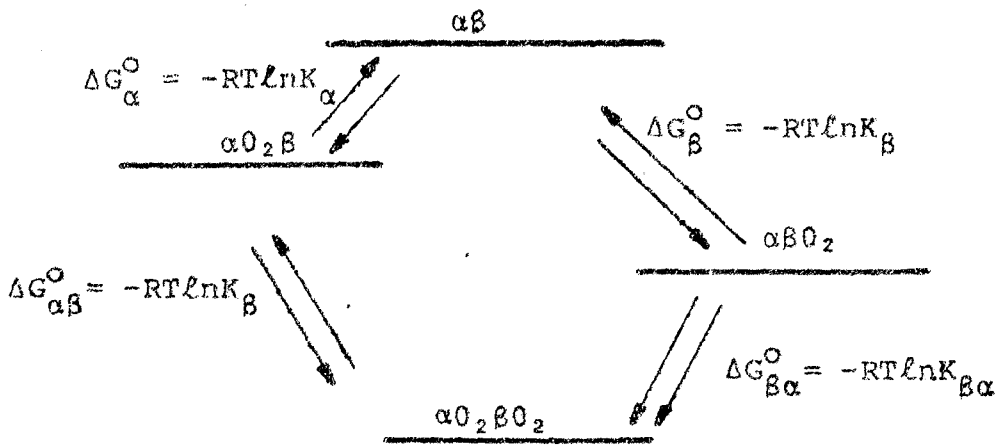
αβ
$\Delta G^{\circ}_{\alpha} = -RT\ell nK_{\alpha}$
$\alpha O_2\beta$
$\Delta G^{\circ}_{\beta} = -RT\ell nK_{\beta}$
$\alpha\beta O_2$
$\Delta G^{\circ}_{\alpha\beta} = -RT\ell nK_{\beta}$
$\Delta G^{\circ}_{\beta\alpha} = -RT\ell nK_{\beta\alpha}$
$\alpha O_2\beta O_2$

FIGURE 5

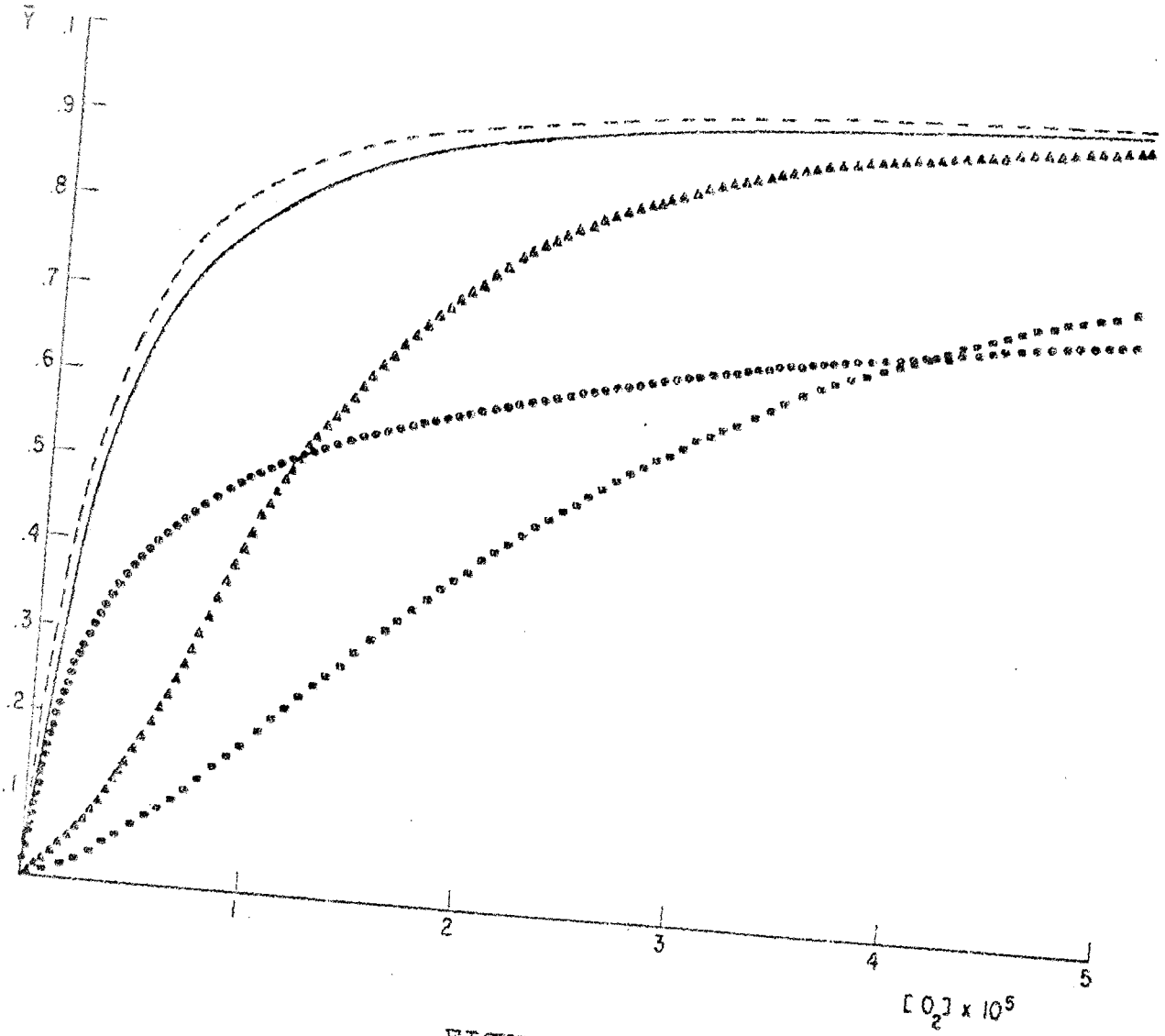

FIGURE 6

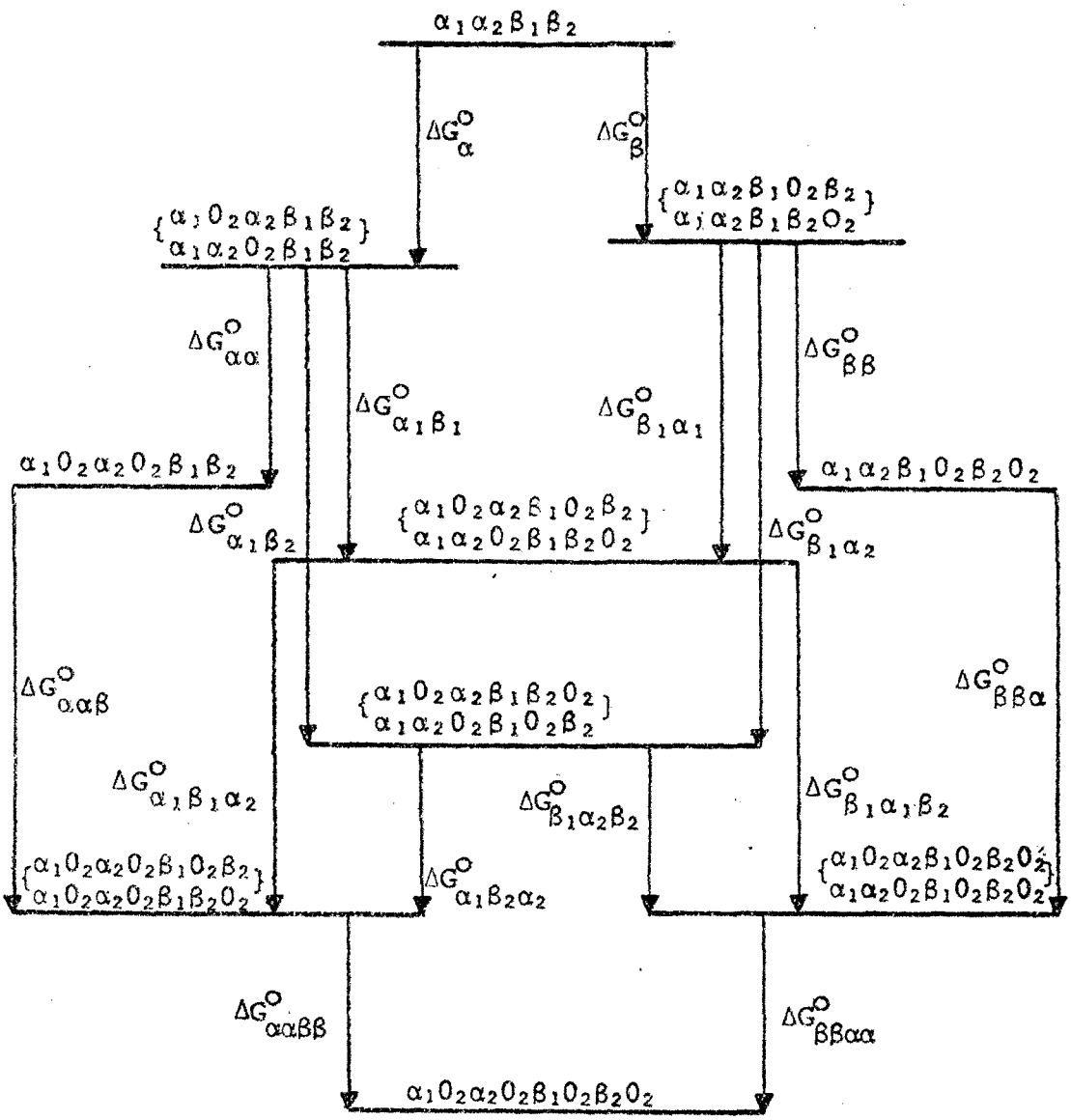
$\alpha_1\alpha_2\beta_1\beta_2$
ΔG^{o}_{α}
ΔG^{o}_{β}
$\{\alpha_1 0_2\alpha_2\beta_1\beta_2\}$ $\alpha_1\alpha_2 0_2\beta_1\beta_2$
$\{\alpha_1\alpha_2\beta_1 0_2\beta_2\}$ $\alpha_1\alpha_2\beta_1\beta_2 0_2$
$\Delta G^{o}_{\alpha\alpha}$
$\Delta G^{o}_{\alpha_1\beta_1}$
$\Delta G^{o}_{\beta_1\alpha_1}$
$\Delta G^{o}_{\beta\beta}$
$\alpha_1 0_2\alpha_2 0_2\beta_1\beta_2$
$\alpha_1\alpha_2\beta_1 0_2\beta_2 0_2$
$\Delta G^{o}_{\alpha_1\beta_2}$
$\{\alpha_1 0_2\alpha_2\beta_1 0_2\beta_2\}$ $\alpha_1\alpha_2 0_2\beta_1\beta_2 0_2$
$\Delta G^{o}_{\beta_1\alpha_2}$
$\Delta G^{o}_{\alpha\alpha\beta}$
$\{\alpha_1 0_2\alpha_2\beta_1\beta_2 0_2\}$ $\alpha_1\alpha_2 0_2\beta_1 0_2\beta_2$
$\Delta G^{o}_{\beta\beta\alpha}$
$\Delta G^{o}_{\alpha_1\beta_1\alpha_2}$
$\Delta G^{o}_{\beta_1\alpha_2\beta_2}$
$\Delta G^{o}_{\beta_1\alpha_1\beta_2}$
$\{\alpha_1 0_2\alpha_2 0_2\beta_1 0_2\beta_2\}$ $\alpha_1 0_2\alpha_2 0_2\beta_1\beta_2 0_2$
$\Delta G^{o}_{\alpha_1\beta_2\alpha_2}$
$\{\alpha_1 0_2\alpha_2\beta_1 0_2\beta_2 0_2\}$ $\alpha_1\alpha_2 0_2\beta_1 0_2\beta_2 0_2$
$\Delta G^{o}_{\alpha\alpha\beta\beta}$
$\Delta G^{o}_{\beta\beta\alpha\alpha}$
$\alpha_1 0_2\alpha_2 0_2\beta_1 0_2\beta_2 0_2$

FIGURE 7