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Hemoglobinopathies Due to Abnormal Functional Properties of Hemoglobin Molecule

Part I. Stable Abnormal Hemoglobins with High Oxygen Affinity and Erythrocytosis

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ABSTRACT. The hemoglobin (Hb) tetramer exists in equilibrium between two quaternary conformations R and T and any alteration that will affect this equilibrium will have a marked effect on the function of hemoglobin. As a rule, it can be stated that any amino acid substitution that would stabilize the Hb tetramer in the oxy conformation will result in a hemoglobin variant with an increased oxygen affinity. Conversely, any amino acid substitution that would favour the deoxy configuration will produce a hemoglobin mutant with decreased oxygen affinity.

Since the discovery of Hb Chesapeake in 1966, more than 130 abnormal hemoglobins with an altered oxygen affinity have been reported. Out of these 77 are stable variants and the rest being unstable hemoglobins. The present review describes the molecular basis of 77 stable variants with an altered oxygen affinity and a comprehensive hematological data which should be of great interest to clinicians has been presented. In many cases, these abnormal hemoglobins manifest their presence either by familial erythrocytosis or clinical cyanosis. The molecular abnormality has been caused by substitution in $\alpha_1\beta_2$ contact, carboxy terminal of globin chains or 2,3 DPG binding site.

Stable abnormal hemoglobins of mutation at $\alpha_1\beta_2$ and $\alpha_1\beta_1$ contacts with high oxygen affinity only are dealt with in the present part of this review.

Key words : Hemoglobinopathies — Hemoglobins with altered oxygen affinity — Erythrocytosis — Cyanosis

The human hemoglobin (Hb) whose main function is to carry oxygen to the tissues is a tetramer of four polypeptide chains (2 α ; 2 non-α). To each globin chain, a heme moiety is attached and the oxygen binds reversibly to the ferrous iron atom in each group. The structure of Hb molecule has been studied extensively by x-ray analysis.[1-3]

The process of oxygenation results in alterations in the tertiary and quaternary structure of Hb, but leaves the primary and secondary helical structures unchanged. The tertiary structure changes slightly with oxygenation, whereas the quaternary structure changes significantly and accounts for the sigmoidal shape of oxygen dissociation curve. This movement is responsible for the

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interaction between the heme groups. The sequence of molecular changes on oxygenation occurs in the following manner. Oxygen is added first to an α chain, then to a second α chain and finally to the two β chains. After the second or third oxygen has been added the quaternary structure changes considerably ; 2,3 DPG which is responsible for stabilizing the deoxygenated Hb is now expelled from the molecule. Movement occurs at the $\alpha_1\beta_2$ and $\alpha_1\beta_1$ contact points, particularly $\alpha_1\beta_2$. Once the change from the deoxygenated to oxygenated configuration is underway, oxygen affinity is greatly increased accounting for the steep rise in the oxygen dissociation curve and oxygen is added to the hemes of remaining β chains.

The oxygen affinity of hemoglobin in the red cells is controlled by several factors. The position and the shape of the curve can be altered by changing the pH (Bohr effect) ; another is temperature and this may be important in delivery of oxygen to the tissues in pyrexia due to infection or excercise or conversely in hypothermia. It is also shown that the glycolytic intermediate 2,3 DPG bind to hemoglobin and lowers its oxygen affinity. The release of oxygen by the red cells in the presence of 2,3 DPG is favoured by decrease in pH and increase in temperature and vice versa.

The process of oxygen delivery to the tissues takes place in three main stages. The first is the loading of oxygen in the lungs which depends upon mainly on the composition of inspired air and pulmonary function. The second and the third stages are the carriage of oxygen by the blood and its release to the tissues. These three stages of oxygen transport however, can be affected by the hemoglobin level, the oxygen affinity of the blood, and blood flow through the tissues.

The Hb tetramer exists in equilibrium between two quaternary conformations R and T. When normal hemoglobin is fully deoxygenated, it is in T state. In this conformation Hb has relatively low affinity for oxygen and heme ligands and relatively high affinity for allosteric affectors such as Bohr protons and 2,3 DPG. Conversely, normal oxyhemoglobin exists almost exclusively in the R state. In this conformation, it has relatively high affinity for heme ligands such as oxygen and low affinity for Bohr protons and 2,3 DPG. A structural alteration which will affect the equilibrium between R and T states is expected to have a marked effect on Hb function. Perutz and his associates[1–3] have demonstrated that oxygen affinity is altered when amino acid substitution affects oxygen binding by :

i) Changing the conformation of heme environment,
ii) Altering the binding of H atoms (Bohr effect) or 2,3 DPG,
iii) Shifting the allosteric equilibrium between the R and T state of Hb molecule.

Thus a specified amino acid substitution would decrease the stability of the T structure, the transition to R state will occur at an earlier state in ligation and the Hb will have an increased oxygen affinity and decreased heme-heme interaction. Abnormal hemoglobins with increased oxygen affinity, under the physiological condition will unload less oxygen during their passage through capillaries. The patients carrying such hemoglobins will often have an increased Hb levels and red cell counts to compensate for the fraction of poorly functioning Hb that they carry. Now that much of the structure of the oxy-and deoxy Hb molecule has been elaborated by Perutz and his colleagues,[1–3] it is possible in

case of many abnormal hemoglobins to interpret in stereochemical terms the effect of an amino acid substitution on oxygen affinity. As a working rule, it can be stated that any amino acid substitution that tend to stabilize the tetramer in the oxy configuration will result in increased oxygen affinity. Conversely, any amino acid substitution that favours the deoxy conformation will result in a hemoglobin with decreased oxygen affinity.

Hemoglobinopathy is a term used to describe a condition in which an abnormal hemoglobin is produced due to substitution of one or more amino acids in any part of normal hemoglobin molecule. But there are some other mechanisms by which a Hb variant can result. These variants could be both stable or unstable with normal or altered functional properties depending on the type and site of amino acid substitution in the molecule. When this amino acid substitution impairs ability to release oxygen at the tissue level ; the mutant is known as increased oxygen affinity hemoglobin, causing the shift of the whole blood oxygen dissociation curve to the left. Often, but not always, an erythrocytosis is associated with high oxygen affinity hemoglobinopathies ; the condition which is considered to be secondary to and caused by tissue hypoxia. The resulting anoxia stimulates compensatory erythropoietin production invoking the hemostatic mechanism to produce more red cells in order to transport a sufficient amount of oxygen. Under the physiological conditions of pH (7.4) and temperature (37°C), an increased oxygen affinity hemoglobin is always associated with low P_{50} value (12-18 mmHg) as compared to normal (27±1 mmHg). Conversely it is true with those abnormal hemoglobins with decreased oxygen affinity which produce cyanosis in many cases and oxygen dissociation curve is shifted to the right when whole blood from an affected patient is examined. This shift reflects the decrease in oxygen affinity, the rise in oxygen saturation for any increase in partial pressure of oxygen being less than that found in normal adult blood.

In 1966, Charache *et al.*[4] described a patient with unexplained erythrocytosis associated with a hemoglobinopathy. His Hb was 19.9 g dl ; with an abnormal Hb electrophoretic band and P_{50} of 20 mmHg. The further investigation suggested the cause of the patient's erythrocytosis was a secondary compensation to a primary defect in oxygen unloading. The abnormal hemoglobin was purified and structural analysis showed that the propositus was carrier of abnormal α chain mutant, Hb Chesapeake (α 92 Arg→Leu).[5]

At present nearly 500 human hemoglobin variants of the α, β, γ and δ chains have been reported from all over the world, the details of which are regularly published in HEMOGLOBIN[6,7] and further informations are available from International Hemoglobin Information Center (IHIC), Medical College of Georgia, Augusta, GA 30912, U.S.A. Of these reported, nearly one fourth are the variants (both stable and unstable) with an altered oxygen affinity (Table 1). Of the total 134 variants with abnormal functional properties 77 are stable mutants belonging to α, β and δ chains. 43 are unstable hemoglobins and the remaining 14 have arisen through different mechanisms such as deletion of certain amino acids, fusion of two normal chains, elongation of the chain, or mutations at more than one point in the same polypeptide chain. The figures 1 and 2 denote the position of α and β chain stable mutants in their respective helices. These mutants are mostly inherited as an autosomal dominant and all the subjects reported so far (except Hb Abruzzo) are heterozygous for

TABLE 1. Hemoglobin variants with an altered oxygen affinity

Variants	α	β	γ	δ
Stable	22	54	0	1
Unstable	7	36	0	0
			Subtotal :	120
		Others (deletion, fusion, extended chain residue, two point mutation, etc.)		14
			Total :	134

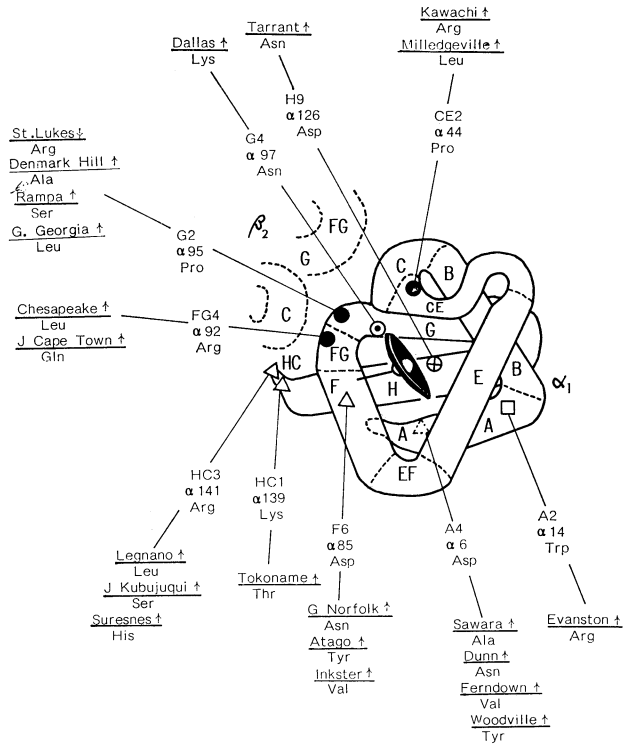

Fig. 1. α chain stable mutants of hemoglobins with altered oxygen affinity. A,B,C, ···, H: helices. CE, EF, FG: nonhelices. HC: C- or carboxy terminus of α chain.
● $\alpha_1\beta_2$ contact ⊕ $\alpha_1\beta_1$ contact ⊙ Heme contact □ Internal
△ External ↑ high oxygen affinity ↓ low oxygen affinity

the specific condition. The discovery of these inherited hemoglobin variants with an increased or decreased oxygen affinity had been an important stimulus to the study of the mechanism involved during oxygenation and deoxygenation process. The effect of progressive oxygenation results in the alterations in the distances between the iron atoms ; and the changes which are more significant concern those between the iron atoms of the β chain. These are in total of eight bands which are responsible for keeping the Hb in the deoxy state and rupture of these converts the spring loaded deoxy Hb (T) into relaxed (R) oxy Hb.

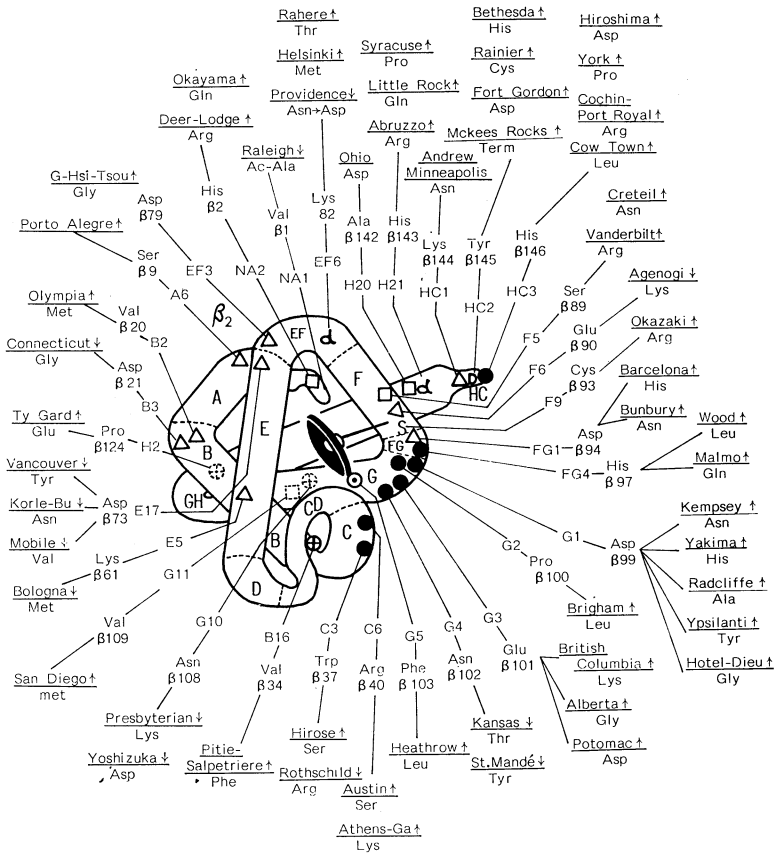

Fig. 2. β chain stable mutants of hemoglobins with altered oxygen affinity. A,B,C,···, H: helices. NA: N- or amino terminus of β chain. HC: C- or carboxy terminus of β chain. CD, EF, FG, GH: non helices.
● $\alpha_1\beta_2$ contact ⊕ $\alpha_1\beta_1$ contact ⊙ Heme contact □ Internal
△ External ↑ high oxygen affinity ↓ low oxygen affinity
D (HC2) deoxyl d 2-3 DPG binding S Active SH radical

In this conformation, the successive oxygenation of the subunits leads to a concurrent shift in contacts between the α and β chains.

Most of the hemoglobin variants with an altered oxygen affinity have an amino acid substitution in areas which are critically important to the function of hemoglobin molecule e.g. $\alpha_1\beta_2$ contact, the carboxy terminal of the β chain or 2,3 DPG binding site. In addition to that there are many other mutants which affect the different contacts of Hb molecule. All these variants will be discussed under their appropriate sections of the text.

HEMOGLOBIN MUTANTS WITH AN INCREASED OXYGEN AFFINITY

The majority of the hemoglobins with an increased oxygen affinity reported so far, either have the substitution at the $\alpha_1\beta_2$ interface, or carboxyl terminal of the β chain, the regions which are crucially important to the function of

hemoglobin molecule. An amino acid substitution affecting the residues involved in 2,3 DPG indirectly can also cause a high oxygen affinity. The $\alpha_1\beta_2$ contact is of the clinical importance. It is responsible for heme-heme interaction, which is vital for oxygen transport. As the oxygen affinity of Hb in the red cells is largely determined by the equilibrium between the high and low affinity forms of hemoglobin and any structural change in the Hb can affect this, it is not surprising that 24 abnormal hemoglobins with increased oxygen affinity have a substitution at the $\alpha_1\beta_2$ interface.

As stated earlier, that $\alpha_1\beta_2$ contact is important, it is at this contact that movement primarily occurs between the subunits ($\alpha_1\beta_2$) during oxygenation, hence its disruption would favour either of the conformation of Hb molecule which would be reflected either in the form of lower or increased oxygen affinity. In case of hemoglobins with high oxygen affinity this may result either from stabilization of the oxy conformation or destabilization of the deoxy conformation.

The stable hemoglobin variants with an increased oxygen affinity can be broadly subdivided into the following groups and nearly half of them are associated with a clinical condition known as familial erythrocytosis :

1. Hemoglobin variants involving $\alpha_1\beta_2$ contact producing an erythrocytosis,
2. Hemoglobin variants *not* involving $\alpha_1\beta_2$ contact, but cause an erythrocytosis,
3. Hemoglobin variants affecting carboxy terminal of polypeptide chain and also producing erythrocytosis,
4. Hemoglobin variants with increased oxygen affinity involving $\alpha_1\beta_2$ contact but without erythrocytosis,
5. Other hemoglobin variants affecting different contacts of the globin chains but without any clinical manifestations.

1. HEMOGLOBIN VARIANTS INVOLVING $\alpha_1\beta_2$ CONTACT PRODUCING AN ERYTHROCYTOSIS

The structural alterations at the $\alpha_1\beta_2$ contact can result in drastic changes in the functional properties of Hb molecule. At present there are 15 stable hemoglobin mutants in which $\alpha_1\beta_2$ contact has been affected (Table 2) and are responsible for producing a well-known clinical condition termed as familial erythrocytosis. On the basis of the information available, it is possible to explain in stereochemical terms the effect of amino acids substitution in some variants on the oxygen affinity of the hemoglobin molecule. As a general rule any amino acid that would stabilize the Hb tetramer in the oxy conformation will produce a variant with raised oxygen affinity.

Substitution of amino acid residue at position 44 of the α chain has been reported in two variants namely Hb Milledgeville α 44 (CE2) Pro→Leu[8] and Hb Kawachi α 44 (CE2) Pro→Arg.[9] Hb Milledgeville was found in a black American. The oxygen affinity of whole blood from the affected patient showed a marked increase (P_{50}= 11-15 mmHg) and the abnormality was associated with mild erythrocytosis and the similar tendency was found in a Japanese patient with Hb Kawachi. In both Hb Milledgeville and Hb Kawachi, a prolyl residue α 44 (CE2) which is common to all mammalian hemoglobins and is a part of the interface was replaced, In the oxy state, Pro

TABLE 2. Hemoglobin variants with increased oxygen affinity causing familial erythrocytosis involving $\alpha_1\beta_2$ contact

S. Variant No. Substitution	Position in molecule	RBC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Retics (%)	Abn. Hb(%)	Race	Elect. Mobility	O_2 Affinity	n*	Bohr effect	Ref.
1. Milledgeville α44(CE2)Pro→Leu	E, deoxy	6.1	18.1		85			2.7		Black American	Like A	Increased	↓	Normal	8
2. Kawachi α44(CE2)Pro→Arg	Same	5.6	15.4	46	81	27	33	1.4	4.1	Japanese	Between A_2 and A	Increased			9
3. J-Cape Town α92(FG4)Arg→Gln	E		16.6	52			32	0.8	48.0	South African Black	Faster than A	Increased	Normal	Normal	12,13
4. Chesapeake α92(FG4)Arg→Leu	Same	6.4	19.9	58	91	31	34		34.9	German Irish	Faster than A	Increased	↓	Normal	4,5
5. Malmö β97(FG4)His→Gln	E	5.2–7.0	15.3–21.8	47–65					48.0	Swedish	Like A	Increased	↓	Norma l	14–16
6. Wood β97(FG4)His→Leu	Same	6.3–6.8	18.6–21.5	64–67					50.0	Swedish	Faster than A	Increased	↓		17
7. Kempsey β99(G1)Asp→Asn			21.3	63					37.0	Irish	Like F	Increased	↓	Reduced	19–21
8. Yakima β99(G1)Asp→His		5.0–7.3	16.5–22.9	45–55					38.0	American	Faster than A	Increased	↓	Normal	21,23
9. Ypsilanti β99(G1)Asp→Tyr		6.6	19.0	63					40.0	Black American	Asym. Hybrid	Increased			24
10. Radcliffe β99(G1)Asp→Ala		6.6	18.1	56	85	27	32		47.0	English	Like F	Increased	Absent	Reduced	25
11. Hotel-Dieu β99(G1)Asp→Gly		6.4	18.9	63	100			1.8	48	French	Like F	Increased	↓		26
12. Brigham β100(G2)Pro→Leu	Facing IC; deoxy	6.1	20.0	59				3.0	50.0	American	Like A	Increased	↓	Normal	27
13. British Columbia β101(G3)Glu→Lys		6.8	16.8	49	72	25	34		54.1	East Indian	Between S and A	Increased	↓	Normal	33
14. Alberta β101(G3)Glu→Gly		6.2	19.8	61	97	31	32		45.0	Canadian	Slower than S	Increased	↓	Reduced	34
15. Potomac β101(G3)Glu→Asp			19.0					1.4		American	Like A	Increased		Normal	35

* Heme–heme interaction

residue does not participate in interchain contact formation,[10] but in the deoxy form, part of the α CE region rotates to a position where it protrudes into a crevice formed by FG region, bringing α 44 prolyl residue to within 4 Å of β 97 (FG4) His.[11] The amino acid substitution of α 44 (CE4) Pro either by Leucyl residue as in Hb Milledgeville or by Arginyl as in Hb Kawachi will result in disruption on the $\alpha_1\beta_2$ contact surface, because in both cases, Leucyl, δ-Methyl and Arginine guanidyl group will block the space usually occupied by the imidazole group β 97 (FG) His, a residue which plays an important role in stabilizing deoxy form of Hb. The resulting destabilization of deoxy conformation would tend to shift the equilibrium towards oxy form giving rise to above hemoglobins with an increased oxygen affinity.

Charache and co-workers[4,5] were the first to describe a hemoglobinopathy with increased oxygen affinity and erythrocytosis—Hb Chesapeake 92 (FG4) Arg →Leu.[4,5] In the same year Hb J-Capetown α92 (FG4) Arg→Gln[12,13] with similar clinical manifestations was reported in a South African. A loss of the probable salt bridge [between Arg 92 α_2 (FG4) and Glu 43 β 2 (CD2)] in the quaternary deoxy structure, due to substitution of either leucine or glutamine for arginine probably accounts for the increased oxygen affinity of both Hb Chesapeake and Hb J-Cape Town.

Three amino acid substitution has been reported at position 97 of the β chain ; this corresponds with position 92 of the α chain. These are in Hb Malmö β 97 (FG4) His→Gln,[14–16] Hb Wood β 97 (FG4) His→Leu[17] and Hb Nagoya β 97 (FG4) His→Pro.[18] Both Hb Malmö and Hb Wood cause erythrocytosis but the propositus with Hb Nagoya (an unstable Hb) suffer from chronic hemolytic crisis as the proline substitution would disrupt the helical formation causing the instability of Hb molecule and shortening the life span of erythrocyte. All the three hemoglobin variants, namely Hbs Malmö, Wood and Nagoya have an increased oxygen affinity, but it is not clear that why these substitutions alter the oxygen affinity. The results of oxygen equilibrium study suggest that all these variants are always fixed in the oxy quaternary conformation. His (FG4) β 97 which is located at the $\alpha_1\beta_2$ interface, its substitution by another amino acid probably would reduce the stability of deoxy structure relative to that of the oxy structure.

There are five different substitutions that has been described at β 99 (G1) Asp, namely Hb Kempsey β 99 (G1) Asp→Asn[19–21] ; Hb Yakima β 99 (G1) Asp→His[22,23] ; Hb Ypsilanti β 99 (G1) Asp→Tyr[24] ; Hb Radcliffe β 99 (G1) Asp→Ala[25] and Hb Hotel-Dieu β 99 (G1) Asp→Gly.[26] Asp β 99 (G1) is an amino acid which participate in the contacts which is characteristic for deoxy conformation and is bonded to the tyrosyl residue α 42 (C7) only in the deoxy structure. Substitution of this asparty residue β 99 by an asparaginyl residue either in Hb Kempsey or by a histidyl residue in Hb Yakima or by a tyrosyl residue in Hb Ypsilanti or by an alanyl residue in Hb Radcliffe or by a glycine in Hb Hotel-Dieu would cause this important bond to be absent resulting in a disturbance of quaternary structure. These variants thus would favor the oxy conformation and as a result will have an increased oxygen affinity. All the above hemoglobin variants are with virtually complete loss of heme-heme interaction. In case of Hb Kempsey and Hb Radcliffe alkaline Bohr effect is also reduced. Impairment of the Bohr effect is probably a reflection of the

decreased ability of deoxygenated forms of these hemoglobins to assume the T conformation.

Like β 99 (G1) Asp, the prolyl residue Pro β 100 (G2) also participate in the contacts which are characteristic for the deoxy conformation. In Hb Brigham β 100 (G2) Pro→Leu,[27] the Pro β 100 (G2) which is part of $\alpha_1\beta_2$ contact in deoxy Hb forms a contact with Thr 38 α (C3) in the deoxy form ; replacement of this prolyl residue by a leucyl residue as in Hb Brigham alters this contact, because the larger size of the leucyl side chain would interfere with the hydrogen bond between neighbouring Asp 99 β (G1) and Tyr 42 α (C7) in the deoxy state of hemoglobin. Four different amino acid substitutions[28–31] have been reported for α 95 which corresponds with position 99 of the β chain. These are in Hb G-Georgia, Hb Rampa, Hb Denmark Hill and Hb St. Lukes ; neither of these variants cause erythrocytosis.

Residue β 101 (G3) Glu normally participates in the $\alpha_1\beta_2$ contact. It also interacts with α 94 Asp in the deoxy Hb which is absent in the oxy Hb. Moreover, it forms a hydrogen bond to Asp 99 (G1) of the same β chain and interact with Arg 104 (G6) of the β chain.[10] Hb Rush β 101 (G3) Glu→Gln[32] an unstable hemoglobin with normal oxygen affinity was found to be first example of substitution of this residue. In contrast to that three stable abnormal hemoglobins, Hb British Columbia β 101 (G3) Glu→Lys,[33] Hb Alberta β 101 (G3) Glu→Gly[34] and Hb Potomac β 101 (G3) Glu→Asp[39] with increased oxygen affinity and erythrocytosis in the carriers have been reported. In Hb British Columbia or Hb Alberta, the destabilization of the deoxy conformation may result from introduction of excessive positive charge or water into the interior of the molecule. The same theory of destabilization of deoxy structure has been put forward for Hb Potomac. The side chain of the normal glutamic acid residue is only 3.5 Å from Asp (G1) in the deoxy conformation. When an aspartyl residue is substituted at G3 β, the carboxylated oxygens come within 2.5 Å, close enough to cause strong repulsive forces and destabilizing the deoxy structure of hemoglobin molecule.

2. *VARIANTS NOT INVOLVING $\alpha_1\beta_2$ CONTACT BUT CAUSE AN ERYTHROCYTOSIS*

The group includes 12 abnormal hemoglobins with an increased oxygen affinity and also produce erythrocytosis in the carriers. Some of these variants involve either $\alpha_1\beta_2$ contact or 2,3 DPG binding site. In Hb Olympia β 20 (B2) Val → Met[33] valine residue which is located at the surface of molecule has been replaced by the sulfur containing methionine. There is no special function attributed to residue Met β 20 (B2) in the normal hemoglobin and its replacement should not affect the functional properties of the hemoglobin molecule, but for some unknown reasons which are not clearly understood, the variant is associated with high oxygen affinity. It is just possible this surface mutation of β 20 Val→Met might have some effect on the neighbouring or distal residues, however, no x-ray analysis of the variant is available to support this view.

Hb Pitie-Salpetriere β 34 (B16) Val→Phe[34] and Hb Ty Gard β 124 (H12) Pro→Gln[35] both involve $\alpha_1\beta_1$ contact and are stable variants. Most of high

TABLE 3. Variants not involving $\alpha_1\beta_2$ contact but cause erythrocytosis

S. Variant No. Substitution	Contact	Position in molecule	RBC (10^{12}/L)	Hb (g/dl)	PCV (%)	MCV (fl)	MCH (pg)	MCHC (g/dl)	Retics (%)	Abn. Hb(%)	Race	Elect. Mobility	O_2 Affinity	n*	Bohr effect	Ref.
1. Olympia β20(B2) Val→Met		E	6.9	20.7	62	90	30	33		40.0	American	Like A	Increased	Normal	Normal	33
2. Pitie-Salpetriere β34(B16) Val→Phe	$\alpha_1\beta_1$	SP	6.1	20.0	61	99					French	Like A	Increased			34
3. Rahere β82(EF6) Lys→Met	2,3 DPG binding site	SCC	6.0	19.0	57	95	32	33	0.4	48	English	Like A	Increased		Normal	37
4. Helsinki β82(EF6) Lys→Met	Same	Same		19.1	54				3.9		Finnish	Faster than A	Increased	Normal	Reduced	38
5. Creteil β89(F5) Ser→Asn		I	7.0	22.0	65	94	34	32		50.0	French	Like A	Increased	↓	Decreased	41
6. Vanderbilt β89(F5) Ser→Arg		Same	7.5	21.8	64						American	Like A	Increased			42
7. Barcelona β94(G1) Asp→His	SB to His HC3(146)	E deoxy								37.0	Spanish		Increased	↓	Normal	43
8. Banbury β94(G1) Asp→Asn	Same	Same	4.9	12.8		84	28			38.0	Italian	Between A and S	Increased	↓	Reduced	44
9. Heathrow β103(G5) Phe→Leu	Heme	I		21.0	66			32	1.2		English	Like A	Increased	↓	Normal	45
10. San Diego β109(G11) Val→Met	Close to $\alpha_1\beta_1$	I		18.0	52						Filipino	Like A	Increased	↓	Normal	46
11. Ty Gard β124(H2) Pro→Gln	$\alpha_1\beta_1$	E	6.0	19.2	54	88	31	35		40.0	French	Like A	Increased	Normal	Normal	35
12. Ohio β142(H20) Ala→Asp		CC	6.1	18.6	54	88	30	34		48.0	Scotch	Between A and J	Increased	↓	Reduced	47

* Heme-heme interaction

oxygen affinity hemoglobins have their amino acid substitution localized in the $\alpha_1\beta_2$ contact, but there are few examples of substitution in the $\alpha_1\beta_1$ contact as the structural alterations at this contact not only can affect the stability but also can drastically alter the functional properties of the molecule. In Hb Pitie-Salpetriere, a hydrogen bond which β 34 Val (B16) forms with the residues α 122 His (H5) and α126 Asp (H9) is affected. Proline β 124 is an external but invariant residue in all α and non α chains, the substitution of Gln for Pro at β 124 is probably responsible for the physiological consequences of Hb Ty Gard which are noticeable in term of the increase in erythrocyte mass. Another substitution has been reported for this residue ; the variant is Hb Khartoum β 124 Pro→Arg[36] but it is an unstable hemoglobin.

2,3 DPG plays an important role in stabilizing the deoxy conformation of Hb and an amino acid substitution affecting the residues involved in 2,3 DPG binding can indirectly cause a raised oxygen affinity. Three different substitutions for the residue β 82 (EF6) Lys, a binding site for 2,3 DPG has been reported. These are Hb Rahere β 82 (EF6) Lys→Thr[37] ; Hb Helsinki β 82 (EF6) Lys→Met[38] and Hb Providence β 82 (EF6) Lys→Asn→Asp.[39,40] Interestingly Hb Providence exists in two forms in vivo. Hb Providence (Asn) apparently has arisen from a single genetic change in codon that substitute Asn for Lys and the second form Hb Providence (Asp) is probably result of a partial in vivo deamidation. Hb Providence has decreased oxygen affinity in contrast to Hb Rahere and Hb Helsinki which not only have raised oxygen affinity but normal heme-heme interaction and alkaline Bohr effect.

In Hbs Creteil β 89 (F5) Ser→Asn[41] and Vanderbilt β 89 (F5) Ser→Arg[42] an internal amino acid is replaced. β 89 (E5) Ser is not involved either in heme binding, ligand coordination or any $\alpha\beta$ contacts and the exact mechanism responsible for an altered functional properties of these variants is not clear until a detailed x-ray analysis is performed. It is likely that the replacement of serine by asparagine as in Hb Creteil or by arginine as in Hb Vanderbilt most probably will disturb the conformational arrangement of the tyrosyl residue β 145 (HC2) thus inhibiting the molecules existing in a normal deoxy conformation.

A loss of an important interchain salt bridge Asp β 94 (FG1)↔His β 143 (HC3) which is a prerequisite for the full expression of the alkaline Bohr effect and heme-heme interaction has been reported in both Hb Barcelona β 94 (FG1) Asp→His[43] and Hb Banbury β 94 (FG1) Asp→Asn.[44]

Hb Heathrow is the only abnormal hemoglobin which is associated with erythrocytosis and involves a substitution in the heme pocket. The substitution in Hb Heathrow β 103 (G5) Phe→Leu[45] affects phenylalanine, a residue which in the deoxy conformation supports the heme plate when it has more upright position. The replacement of the phenylalanyl residue by the smaller leucyl residue removes this support ; as a result, the molecule tends to remain in oxy conformation. Hb San Diego β 109 (G11) Val→Met[46] is another electrophoretically silent variant. The valine β 109 is not directly involved in either of the contacts between the globin chains, but the substituted methionyl residue appears to lie close to the border of the heme crevice. Its proximity to Asn β 108 (G10) and Cys β 112 (G14) which participate in the $\alpha_1\beta_2$ contact suggests that this contact may be affected in Hb San Diego.

Hemoglobin Ohio β 142 (H20) Ala→Asp[47] was found in three members of a Caucasian family, all of whom had an erythrocytosis. The variant exhibited an increased oxygen affinity, reduced Bohr effect and decreased heme-heme interaction. The abnormal functional properties of Hb Ohio can be explained by the proximity of the substituent β 142 residue both to His β 143 which is involved in the 2,3 DPG binding site and to the critical carboxy terminal region of the β chain which participates in the stabilization of the deoxy (T) conformation of hemoglobin molecule. The substitution of charged aspartyl residue for non polar analyl residue probably would alter the conformation of the carboxy terminal. (To be continued to the second part.)

REFERENCES

1) Perutz, M.F. : Relation between structure and sequence of haemoglobin. Nature (London) **194** : 914-917, 1962
2) Cullis, A.F., Muirhead, H., Perutz, M.F., Rossmann, M.G. and North, A.C.T. : The structure of haemoglobin. IX. A three-dimensional Fourier synthesis at 5.5 Å resolution : description of the structure. Proc. R. Soc. Lond. Ser. A **265** : 161-187, 1962
3) Perutz, M.F. : Stereochemistry of cooperative effects of haemoglobin. Nature (London) **228** : 726-739, 1970
4) Charache, S., Weatherall, D.J. and Clegg, J.B. : Polycythemia associated with a hemoglobinopathy. J. Clin. Invest. **45** : 813-822, 1966
5) Clegg, J.B., Naughton, M.A. and Wetherall, J.B. : Abnormal human haemoglobins. Separation and characterization of the α and β chains by chromatography, and the determination of two new variants, Hb Chesapeake and Hb J (Bangkok). J. Mol. Biol. **19** : 91-108, 1966
6) Wrightstone, R.N. : Variant list. Hemoglobin 8 : 241-323, 1984
7) Wrightstone, R.N. : Variant list. Hemoglobin **9** : 227-233, 1985
8) Honig, G.R., Vida, L.N., Shamsudin, M., Mason, R.G., Schlumpf, H.W. and Luke, R.A. : Hemoglobin Milledgeville (α44 (CD2) Pro→Leu) a new variant with increased oxygen affinity. Biochim. Biophys. Acta **626** : 424-431, 1980
9) Harano, T., Harano, K., Ueda, S., Shibata, S., Imai, K., Ohba, Y., Shinohara, T., Horio, S., Nishioka, K. and Shirotani, H. : Hemoglobin Kawachi [α44 (CE2) Pro→Arg] : A new hemoglobin variant of high oxygen affinity with amino acid substitution at $\alpha_1\beta_2$ contact. Hemoglobin **6** : 43-49, 1982
10) Perutz, M.F., Muirhead, H., Cox, J.M. and Coaman, L.C.G. : Three-dimensional Fourier synthesis of horse haemoglobin at 2.8 Å resolution : The atomic model. Nature (London) **219** : 131-139, 1968
11) Fermi, G. : Three-dimensional Fourier synthesis of human deoxyhaemoglobin at 2.5 Å resolution : Refinement of the atomic model. J. Mol. Biol. **97** : 237-256, 1975
12) Botha, M.C., Beale, D., Isaacs, W.A. and Lehmann, H. : Haemoglobin J Cape Town $\alpha_2^{92Arg\rightarrow Gln}\beta_2$. Nature **212** : 792-795, 1966
13) Lines, J.G. and McIntosh, R. : Oxygen binding by Haemoglobin J Cape Town ($\alpha_2^{92Arg\rightarrow Gln}$). Nature **215** : 297-298, 1967
14) Lorkin, P.A., Lehmann, H., Fairbanks, V.F., Berglund, G. and Leonhardt, T. : Two new pathological haemoglobins : Olmsted β 141 (H19) Leu→Arg and Malmö β97 (FG4) His→Gln. Biochem. J. **119** : 68-74, 1970
15) Fairbanks, V.F., Maldonado, J.E., Charache, S. and Boyer, S.H. : Familial erythrocytosis due to electrophoretically undetectable hemoglobin with impaired oxygen dissociation (Hemoglobin Malmö, $\alpha_2\beta_2^{97Gln}$). Mayo Clinic Proc. **46** : 721-727, 1971
16) Boyer, S.H., Charache, S., Fairbanks, V.F., Maldonado, J.E., Noyes, A. and Gayle, E.E. : Hemoglobin Malmö β97 (FG-4) Histidine→Glutamine : A cause of polycythemia. J. Clin. Invest. **51** : 666-676, 1972
17) Taketa, F., Huang, Y.P., Libnoch, J.A. and Dessell, B.H. : Hemoglobin Wood β97 (FG4) His→Leu : A new high oxygen affinity hemoglobin associated with familial erythrocytosis. Biochim. Biophys. Acta **400** : 348-353, 1975
18) Ohba, Y., Imanaka, M., Matsuoka, M., Hattori, Y., Miyaji, T., Funaki, C., Shibata, K.,

Shimokata, H., Kuzuya, F. and Miwa, S : A new unstable, high oxygen affinity hemoglobin : Hb Nagoya or β97 (FG4) His→Pro. Hemoglobin **9** : 11-24, 1985
19) Reed, C.S., Hampson, R., Gordon, S., Jones, R.T., Novy, M.J., Brimhall, B., Edwards, M.J. and Koler, R.D. : Erythrocytosis secondary to increased oxygen affinity of a mutant hemoglobin, Hemoglobin Kempsey. Blood **31** : 623-632, 1968
20) Ogata, R.T. and McConnell, H.M. and Jones, R.T. : Binding of triphosphate spin labels to hemoglobin Kempsey. Biochem. Biophys. Res. Commun. **47** : 157-165, 1972
21) Bunn, H.F., Wohl, R.C., Bradley, T.B., Cooley, M. and Gibson, Q.H. : Functional properties of Hemoglobin Kempsey J. Biol. Chem. **249** : 7402-7409, 1974
22) Jones, R.T., Osgood, E.E., Brimhall, B. and Koler, R.D. : Hemoglobin Yakima : I. Clinical and biochemical studies. J. Clin. Invest. **46** : 1840-1847, 1967
23) Novy, M.J., Edwards, M.J. and Metcalfe, J. : Hemoglobin Yakima : II. High blood oxygen affinity associated with compensatory erythrocytosis and normal hemodynamics. J. Clin. Invest. **46** : 1848-1854, 1967
24) Rucknagel, D.L., Glynn, K.P. and Smith, J.R. : Hemoglobin Ypsilanti characterized by increased oxygen affinity, abnormal polymerization and erythremia. Clin. Res. **15** : 270, 1967
25) Weatherall, D.J., Clegg, J.B., Callender, S.T., Wells, R.M. G., Gale, R.E., Huehns, E.R., Perutz, M.F., Viggiano, G. and Ho, C. : Haemoglobin Radcliffe ($\alpha_2\beta_2$99 (G1) Ala) : A high oxygen-affinity variant causing familial polycythaemia. Br. J. Haematol. **35** : 177-191, 1977
26) Blouquit, Y., Braconnier, F., Galacteros, F., Arous, N., Sorita, J., Zittoun, R. and Rosa, J. : Hemoglobin Hotel-Dieu β99 Asp→Gly (Gl). A new abnormal hemoglobin with high oxygen affinity. Hemoglobin **5** : 19-31, 1981
27) Lokich, J.J., Mahoney, C.W., Bunn, H.F., Bruckheimer, S.M. and Ranney, H.M. : Hemoglobin Brigham ($\alpha_2{}^A\beta_2{}^{100Pro\rightarrow Leu}$). Hemoglobin variant associated with familial erythrocytosis. J. Clin. Invest. **52** : 2060-2067, 1973
28) Huisman, T.H.J., Adams, H.R., Wilson, J.B., Efremov, G.D., Reynolds, C.A. and Wrightstone, R.N. : Hemoglobin G Georgia or $\alpha_2{}^{95Leu(G2)}\beta_2$. Biochim. Biophys. Acta **200** : 578-580, 1970
29) De Jong, W.W.W., Bernini, L.F. and Khan, M.P. : Haemoglobin Rampa : α95 Pro→Ser. Biochim. Biophys. Acta **236** : 197-200, 1971
30) Wiltshire, B.G., Clark, K.G.A., Lorkin, P.A. and Lehmann, H. : Haemoglobin Denmark Hill α95 (G2) Pro→Ala, a variant with unusual electrophoretic and oxygen binding properties. Biochim. Biophys. Acta **278** : 459-464, 1972
31) Bannister, W.H., Grech, J.L., Plese, C.F., Smith, L.L., Barton, B.P., Wilson, J.B., Reynolds, C.A. and Huisman, T.H.J. : Hemoglobin St. Luke's or $\alpha_2{}^{95Arg(G2)}\beta_2$. Eur. J. Biochem. **29** : 301-307, 1972
32) Adams, J.B., Winter, W.P., Tausk, K. and Heller, P. : Hemoglobin Rush [β-101 (G3) Glutamine] : A new unstable hemoglobin causing mild hemolytic anemia. Blood **45** : 261-269, 1974
33) Jones, R.T., Brimhall, B. and Gray, G. : Hemoglobin British Columbia [$\alpha_2\beta_2{}^{101(G3)Glu\rightarrow Lys}$]: A new variant with high oxygen affinity. Hemoglobin **1** : 171-182, 1976
34) Mant, M.J., Salkie, M.L., Cope, N., Appling, F., Bloch, K., Jayalakshami, M., Gravely, M., Wilson, J.M. and Huisman, T.H.J. : Hb Alberta or $\alpha_2\beta_2{}^{101(G3)\ Glu\rightarrow Gly}$, a new high-oxygen affinity hemoglobin variant causing erythrocytosis. Hemoglobin **1** : 183-194, 1976-77
35) Bursaux, E., Blouquit, Y., Poyart, C., Rosa, J., Arous, N. and Bohn, B. : Hemoglobin Ty Gard ($\alpha_2{}^A\beta_2{}^{124(H2)\ Pro\rightarrow Gln}$) : A stable high O_2 affinity variant at the $\alpha_1\beta_1$ contact. FEBS Lett. **88** : 155-159, 1978
36) Clegg, J.B., Weatherall, D.J., Wong, H.B. and Mustafa, D. : Two new haemoglobin variants involving proline substitutions. Nature **22** : 379-380, 1969
37) Lorkin, P.A., Stephens, A.D., Beard, M.E.J., Wrigley, P.F.M., Adams, L. and Lehmann, H. : Haemoglobin Rahere β82 Lys→Thr) : A new high oxygen affinity haemoglobin associated with decreased 2,3-diphosphoglycerate binding and relative polycythemia. Br. Med. J. **4** : 200-202, 1975
38) Ikkala, E., Koskela, J., Pikkarainen, P., Rahiala, E.L., El-Hazmi, M.A.F., Nagai, K., Lang, A. and Lehmann, H. : Hb Helsinki : A variant with a high oxygen affinity and a substitution at a 2,3-DPG binding site (β82 [EF6] Lys→Met). Acta Haematol. **56** : 257-275, 1976
39) Moo-Penn, W.F., Jue, D.L., Bechtel, K.C., Johnson, M.H., Schmidt, R.M., McCurdy, P.R., Fox, J., Bonaventura, J., Sullivan, B. and Bonaventura, C. : Hemoglobin Providence.

A human hemoglobin variant occurring in two forms in vivo. J. Biol. Chem. **251** : 7557-7562, 1976

40) Bonaventura, J., Bonaventura, C., Bullivan, B., Ferruzzi, G., McCurdy, P.R., Fox, J. and Moo-Penn, W.F. : Hemoglobin Providence. Functional consequences of two alterations of the 2,3-diphosphoglycerate binding site at position β82. J. Biol. Chem. **251** : 7563-7571, 1976

41) Thillet, J., Blouquit, Y., Garel, M.C., Dreyfus, B., Reyes, F., Cohen-Solal, M., Beuzard, Y. and Rosa, J. : Hemoglobin Creteil β89 (F5) Ser→Asn : High oxygen affinity variant of hemoglobin frozen in a quaternary R-structure J. Mol. Med. **1** : 135-150, 1976

42) Paniker, N.V., Lin, K-T-D., Krantz, S.B., Flexner, J.M., Wasserman, B.K. and Puett, D. : Haemoglobin Vanderbilt ($\alpha_2\beta_2^{89\text{Ser}\rightarrow\text{Arg}}$) : a new hemoglobin with high oxygen affinity and compensatory erythrocytosis. Br. J. Haematol. **39** : 249-258, 1978

43) Wajcman, H., Aguilar, J.L., Bascompte, I., Labie, D., Poyart, C. and Bohn, B. : Structural and functional studies of Hemoglobin Barcelona ($\alpha_2\beta_2^{94\ \text{Asp(FG1)}\rightarrow\text{His}}$). J. Mol. Biol. **156** : 185-202, 1982

44) Como, P.F., Kennett, D., Wilkinson, T. and Kronenberg, H. : A new hemoglobin with high oxygen affinity Hemoglobin Bunbury : $\alpha_2\beta_2^{[94(\text{FG1})\ \text{Asp}\rightarrow\text{Asn}]}$. Hemoglobin **7** : 413-421, 1983

45) White, J.M., Szur, L., Gillies, I.D.S., Lorkin, P.A. and Lehmann, H. : Familial polycythemia caused by a new haemoglobin variant. Hb Heathrow β103 (G5) Phenylalanine →Leucine. Br. Med. J. **3** : 665-667, 1973

46) Nute, P.E., Stamatoyannopoulos, C., Hermodson, M.A., Roth, D. and Hornung, S. : Hemoglobinopathic erythrocytosis due to a new electrophoretically silent variant, Hemoglobin San Diego (β109 (G11) Val→Met). J. Clin. Invest. **53** : 320-328, 1974

47) Moo-Penn, W.F., Schneider, R.G., Shih, T.B., Jones, R.T., Govindarajan, S., Govindarajan, P.G. and Patchen, L.C. : Hemoglobin Ohio (β142 Ala→Asp) : A new abnormal hemoglobin with high oxygen affinity and erythrocytosis. Blood **56** : 246-250, 1980