Analytical Review

The Molecular Basis of the Pathogenicity of Abnormal Hemoglobins—Some Recent Developments

By Paul Heller

This review of the hemoglobinopathies may be considered a “primer,” limiting itself to the discussion of selected material of importance for the understanding of the molecular and submolecular basis of hemoglobin abnormalities. It will be mainly concerned with the highlights of the present concept of hemoglobin structure, and with the relationship of the molecular abnormality of hemoglobin mutants to their pathogenicity.

Normal Hemoglobin

Hemolysates from the red cells of normal human adults contain Hb A, occasionally designated A0,1 and two minor hemoglobin fractions, fetal (F) hemoglobin and Hb A2.14* Hb F is more resistant to alkali denaturation than either hemoglobins A or A2. Normally, the relative amount of Hb F is 1–2 per cent, depending upon which technic is used for its determination.5,6 and of A2 it is 2–2.5 per cent.1 There is strong evidence that Hb F is not uniformly present in all erythrocytes,7 while Hb A2 probably is.1 Each of the three hemoglobin fractions consists of two pairs of polypeptide chains; one pair (α2) is common to all, the other pair is different in each of the three fractions (β2 in the main component, γ2 in Hb F, and δ2 in Hb A2). Separation of these chains can be accomplished by several technics,9-13 countercurrent distribution yielding the purest fractions.13 The determination of the molecular abnormalities of Hb S and C14 was accomplished by separating the peptides of a tryptic digest of heat denatured whole hemoglobin by a combination of high-voltage electrophoresis and paper chromatography (“finger printing”).14 This method is also applicable to the determination of the peptide pattern of isolated polypeptide chains. Recently, Schroeder and his coworkers15 and Jones15a have developed a column chromatographic technic which is likely to become the method of choice for the analysis of peptides after enzymatic digestion.

Proteolytic enzymes hydrolyze denatured protein with varying degrees of specificity at sites of certain amino acid residues; e.g., the C-terminal residue of all peptides obtained by trypsin digestion is either lysine or arginine. Other proteolytic enzymes attack the molecule at other sites, and by determining the overlapping amino acid sequences in the differently obtained peptides it...
has been possible to reassemble the amino acid sequence of the whole chain. With this technic the primary structure of the polypeptide chains of human hemoglobin has been determined in an amazingly short time. The α-chain contains 141 amino acid residues, the β, γ, and δ-chains, 146. The α and β-chains have a few short identical amino acid sequences (61 residues), while the number of homologous residues in the β and γ-chain is 104.2 Iso-leucine is present only in the γ-chain. The δ-chain is even more similar to the β-chain, differing in probably only 8 of the 146 residues. The chains of human hemoglobin have also been found to have immunologic specificity. It is remarkable that the specific chains of the minor fractions, γ and δ, are more antigenic in experimental animals than the α and β chains, the α chains requiring a most sensitive test system for the demonstration of their antigenicity.25

An increasingly clearer picture of the 3-dimensional structure of hemoproteins has evolved from the x-ray crystallographic studies of sperm whale myoglobin by Kendrew and of horse and human hemoglobin by Perutz. It is extremely difficult to represent the complexity of this 3-dimensional helical structure by a two-dimensional illustration. Figure 1 is the result of such an attempt by Schroeder. The amino-acid sequence of the β-chain is depicted with consideration to its helical configuration. The chain is folded into a spatial arrangement that is complementary to that of the α-chain. Nonhelical portions, visualized in figure 1 as linear straight parts of the chain, alternate with helical portions which are designated by capital letters. The N-terminal 6 amino acids are nonhelical. The asymmetrical proline residues which are present in the nonhelical portions are thought to contribute to the bending of the chain. Cross-binding hydrogen bonds, especially between glycine residues, contribute to the stability of the spatial order. The heme moiety of each chain is situated in the crevice between the E and F helix, near the surface of the molecule, with the heme-iron forming a bond with histidyl in position 92 of the β-chain or position 87 of the α-chain, the so-called proximal histidines. The sixth coordination position of iron, the site of reversible oxygenation, points toward another histidyl residue in position 63 of the β-chain or position 58 of the α-chain, the so-called distal histidines. The 4 polypeptide chains are linked into the over-all quaternary structure by bonds which are likely to be stronger between unequal chains (αβ, αγ, αδ) than between like chains (αα, ββ, γγ). The architectural arrangement of the 2 unequal polypeptide chain pairs, with a heme group buried between two helical portions of each chain, apparently makes heme-heme-interaction possible. This interaction is the sine-qua-non for the sigmoid shape of the oxygen dissociation curve of normal hemoglobin. The mechanism by which such a structural arrangement accomplishes this function is still a challenging mystery, although recent studies have begun to unveil the secret.

Abnormal Hemoglobins

Most abnormal hemoglobins are characterized by the substitution of just one amino acid residue by another in the α- or β chain of the molecule. Since each chain is duplicated in the molecule, the replacement of merely 2 amino
acid residues out of the 574 of the entire molecule is responsible for the abnormal characteristics of the hemoglobin mutants. These abnormalities range from simply a change of the electrical charge of the molecule to severe molecular distortions which lead to a crippling, life shortening disease. These vast differences appear to depend chiefly on the physico-chemical characteristics of the replacing amino acid and the site of the substitution in the chain.

The importance of these factors can be illustrated by several examples: The replacement of the hydrophilic glutamyl residue in the sixth position of the $\beta$-chain by the hydrophobic valyl leads to a severe conformational change in the molecule and sickling results. A lysyl in the same position causes the less severe morphologic alterations of the red cell associated with hemoglobin C disease. On the other hand, a lysyl in place of glutamyl in the neighboring position 7 (Hgb C<sub>Georgetown</sub>) causes a propensity to sickling, while the substitution of the same amino acid residue by glycyl is rather harmless (Hgb C<sub>San Jose</sub>). Tyrosine in position 63 of the $\beta$-chain, normally occupied by histidine, forms a bond with the heme iron and renders it unsuitable for reversible oxygenation (Hb M<sub>Kansai</sub>) whereas arginine in the same position (Hb Zurich) increases the susceptibility of the molecule to denaturation, especially by oxidant drugs such as sulfonamides.

**Hemoglobin S**

The mechanism of sickling has attracted the attention of many investigators and its dependence on the concentration of Hb S in the cell, the $O_2$-tension, the pH and other conditions of the intra- and extracellular milieu has been studied in great detail. However, a basic question has not yet been unequivocally answered: Why does the valyl-glutamyl exchange near the N-terminal of the $\beta$-chain produce tactoid formation of deoxygenated, but not of oxyhemoglobin or methemoglobin? The most satisfactory hypothesis explaining the sickling phenomenon has recently been developed by Murayama. He observed that a concentrated solution of deoxygenated Hb S gelled at 38°C, while it was liquid at 0°C. This finding suggests that intramolecular conformational changes take place during cooling of the solution. Measurements of the optical rotatory dispersion, a very sensitive indicator of intramolecular conformational changes, showed the amplitude of the rotatory dispersion to be greater in the gelled than in the liquid state. Since ring formation is associated with increased optical activity, as is well-known from carbohydrate chemistry, the conclusion seemed permissible that a ring structure was present in deoxygenated Hb S at 38°C, but not at 0°C. Deoxygenated Hb A was liquid at either temperature. Because of the known substitution of the hydrophobic valyl in place of the hydrophilic glutamyl in position 6 of the $\beta$-chain (Val-His-Leu-Thr-Pro-Val-Glu-Lys), Murayama proposed that a hydrophobic

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*These factors are expressed in the shorthand code for abnormal hemoglobins. Normal hemoglobin is written as $\alpha^A_2\beta^A_2$ and the amino acid substitution in the abnormal variants is indicated by an appropriate change of the superscript e.g., Hb S is $\alpha^A_2\beta^{6\text{Val}}_2$ (valyl is the sixth residue of this abnormal $\beta$-chain) or Hb G<sub>Philadelphia</sub> is $\alpha^{68\text{Lys}}_2\beta^A_2$ (the 68th residue of the $\alpha$-chain is lysyl instead of asparagyl in the normal $\alpha$-chain).
Fig. 1.—Schematic presentation of the molecular structure of the β-chain by Schroeder.2 Reproduced with the permission of the author and the publisher. The symbols for the amino acid residues which are different in the γ-chain are enclosed in rectangles. The nonhelical portions of the molecule are represented by straight sequences. Note: position of heme between helix E and F, the sites of proximal and distal histidine, the sites of some mutational substitutions, the closeness of the 2 terminal parts of the chain. For other explanations see text.

bond is formed between the two valyl residues 1 and 6. This would allow cyclization by hydrogen bonding between the carbonyl group of the N-terminal valyl and the amino group of threonyl, thus stabilizing this terminal part of the molecule (fig. 2). This cyclic structure of the terminal part of the molecule can be visualized as a “key” at the surface of the molecule that fits into a complementary part (“lock”) of the α-chain of the neighboring molecule. Such a lock and key arrangement would cause stacking of the molecules in a linear array. Stacking along the long axis of the sickled erythrocytes is also suggested by the finding of birefringence (dichroism) of sickled erythrocytes in polarized light.37

Murayama has recently found that “unsickling” can also be accomplished...
Fig. 2.—Schematic presentation of cyclic structure of terminal six amino acid residues of $\beta$-chain (see fig. 1) of Hb S according to Murayama.\textsuperscript{30}

by exposing sickled cells to propane.\textsuperscript{30} This gas also is capable of liquifying gelled Hb S at 38 C. Murayama visualized that these remarkable effects were due to the formation of strong hydrophobic bonds between propane and the valyls in the first and sixth position of the $\beta$-chain. The “interlocking” of these two residues by Van der Waals forces, which seem responsible for the formation of the terminal ring structure, the “key” to sickling, is broken by the stronger hydrophobic interaction of these valyls with propane. This lock and key hypothesis seems to satisfactorily explain the differences in the intramolecular structure of deoxygenated Hb S at physiologic and low temperatures, and its “unsickling” by propane. It does not, however, explain the differences in the physical characteristics between reduced and oxygenated Hb S because the proposed ring structure at the N-terminal of the $\beta$-chain is not opened by oxygenation. How, then, is the stacking of the molecules reversed by oxygenation? A possible explanation may have been provided by an important discovery of Muirhead and Perutz.\textsuperscript{28} They found, by x-ray crystallography, that the distance between the heme groups of the $\beta$-chains in horse oxyhemoglobin was 7 Angstrom units smaller than in reduced human hemoglobin and concluded that this difference in molecular configuration was the effect of oxygenation. Murayama hypothesized that this change of distance between the $\beta$-chains of the hemoglobin molecule during gas exchange could account for the dependence of sickling on deoxygenation. In the movement of the $\beta$-chains toward each other during oxygenation, the distance from a $\beta$ to an $\alpha$-chain of a neighboring molecule could increase, and the N-terminal ring structure of the $\beta$-chain would be removed from the complementary site of the $\alpha$-chain. Thus, “the key would be pulled out of the lock.”\textsuperscript{30} It is of historical interest that in the first publication reporting the electrophoretic differences between hemoglobins S and A, Pauling, Itano, Singer and Wells\textsuperscript{38} anticipated in some measure Murayama’s hypothesis by comparing the postulated molecular events
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Fig. 3.—Effect of 2.48 M phosphate buffer on hemolysate mixtures. Starch gel electrophoregram of mixtures of (1) A and S, (2) cord blood and S, (3) A and S (redissolved precipitate) (4) cord blood and S (redissolved precipitate). Note coprecipitation of A in A-S mixture and its absence in mixture of cord blood with S.

during sickling with the situation “which very probably exists in antigen-antibody reactions.”

Another human hemoglobin which has been found to sickle on deoxygenation is hemoglobin C_georgetown, which has the electrophoretic mobility of Hb C (α^2β^2γδ^2), but which has been found to have the molecular formula α^2β^2γδ^2.31 Murayama30 has postulated that the loss of the hydrophilic glutamyl residue in this hemoglobin at position 7 leads to an electrostatic bond between the glutamyl at position 6 and the N-terminal valyl. This bond is not broken by cooling or propane and, therefore, Hb C_georgetown does not “unsickle” under these influences. A similar mechanism, but with less traumatic consequences, may be the basis of crystal formation of Hb C especially seen in drying blood smears and occasionally in fresh blood preparations from splenectomized individuals homozygous for Hb C.

Murayama’s hypothesis might explain another phenomenon associated with Hb S, its molecular interaction with other hemoglobins. Hb S is less soluble in buffered solutions of high molarity (e.g., 2.48 M phosphate buffer) than Hb A.37,41 When a mixture of hemoglobins A and S is exposed to such highly concentrated solutions, only part of the Hb A remains soluble. The remainder is coprecipitated with Hb S.41 A similar, but somewhat stronger interaction, occurs between hemoglobins S and C. In a mixture of hemoglobins F and S, however, there is hardly any coprecipitation of Hb F (fig. 3). This phenomenon
of molecular interaction can also be observed when mixtures of Hb S and other hemoglobins are allowed to gel, or when blood smears of individuals heterozygous for Hb S are exposed to concentrated buffer solutions. It is possible that the “key” of the β-chain of Hb S finds complementary “locks” on the α-chain of Hb A, but because of the configurational differences between γ- and β-chains, the α-chain of Hb F may not be complementary to the β-chain of Hb S and its “key” does not fit.

This intermolecular interaction, or its absence, very likely influences the severity of the clinical manifestations associated with the heterozygous state. In the sickle cell trait, the relative proportion of Hb S is less than that of Hb A because in this combination the mutant hemoglobin is synthesized at a lower rate than the allelic normal hemoglobin. However, the molecular interaction of Hb S and Hb A may actually increase the “functional” intracellular concentration of Hb S, thus facilitating sickling and contributing to the noxious effects of the sickle cell trait, which probably occur most frequently in the kidney. In S-C disease, the erythrocytes have a greater propensity toward sickling than could be expected from the measurable proportion of Hb S, which is usually approximately 50 per cent. Here, the intermolecular interaction may be responsible for the intraerythrocytic crystals which, according to Diggs and Bell, are characteristic, pathognomonic angular structures which distort the shape of the erythrocytes. Such distorted red cells may have a chronic injurious effect upon the walls of small blood vessels, especially in the eyes and bones. In the adult, a particularly common sequel of this disease is aseptic necrosis of the head of the femur.

The lack of molecular interaction between fetal hemoglobin and Hb S is probably the main reason for the clinical mildness of an abnormality characterized by the presence of approximately 70 per cent Hb S and 30 per cent Hb F in all erythrocytes. Despite the high proportion of Hb S, this condition is clinically no worse than the sickle cell trait. Individuals with this abnormality are heterozygotes for Hb S and an anomaly which has been designated “hereditary persistence of fetal hemoglobin.” This uncommon and usually asymptomatic abnormality has acquired great theoretical importance and it has stimulated the development of several hypotheses regarding the action and interaction of genes in the determination of the synthesis of the polypeptide chains of hemoglobin. It also has provided a large part of the basis for the concept of “controller gene disease.”

It is beyond the scope of this review to discuss these very important hypotheses, except for those points which help to explain the genetic mechanism governing the distribution of fetal hemoglobin in the red cell population. They are here presented in a highly simplified manner: the structural gene which determines the primary structure, the amino-acid sequence, of a polypeptide chain is thought to be part of the “operon,” which also contains an operator gene. The latter is assumed to be under the inhibiting influence of a regulator gene. In this hypothetical concept, interaction of the operator and regulator genes governing the production of the polypeptide chains of hemoglobin could permit the synthetic activity of the β and δ genes and that of the γ genes to occur in a reciprocal manner: when the production of β and δ chains is
"switched on" toward the end of the gestational period, that of \( \gamma \) chains is turned off. A few clones of normoblasts retain their capacity for \( \gamma \) chain production throughout life; they may be stimulated into increased proliferation by a still highly speculative feedback mechanism, possibly emanating from the stress of certain diseases, such as thalassemia or sickle cell anemia. It is of interest in this regard that the bulk of the increased fetal hemoglobin in these diseases is found in a separate erythrocyte population.\(^7\) When there is, however, a mutational alteration in the operator gene which deprives it of its normal function to initiate the synthesis of \( \beta \) and \( \delta \) chains, all hemoglobin producing cell clones are affected and continue to manufacture \( \gamma \) chains. If both chromosomes carry the abnormal gene, the erythrocytes contain exclusively fetal hemoglobin. In the more common heterozygote the structural gene of the allelic operon is either normal, resulting in the A-F phenotype, or a mutant, e.g., Hb S, in which case the erythrocytes of the carrier contain approximately 70 per cent Hb S and the remainder is fetal hemoglobin together with a less than normal amount of Hb A\(_2\). As previously stated, individuals with the S-F anomaly are usually as asymptomatic as persons with the sickle cell trait. In the latter, the sum of Hb S and the interacting part of Hb A in each cell might equal that of Hb S in the erythrocytes of the S-F anomaly, where no interaction occurs. These considerations suggest that higher relative proportions of Hb S than those usually found in the sickle cell trait can be well tolerated, and may not cause significant shortening of the life span of the cell. The possibility, however, that in addition to the absent molecular interaction with Hb S, fetal hemoglobin exerts a protective influence on the molecular stability of other hemoglobins or on the integrity of the cells cannot be excluded.

Hemoglobin Mutants with Disturbed Heme-Oxygen Relationship

In this species of abnormal hemoglobins, strategically located amino-acid substitutions with particular molecular characteristics are responsible for disturbances of the normal function of heme.\(^{54,56}\) The designation "Hb M" was suggested by Singer\(^{57}\) for the abnormal methemoglobin which Hörlein and Weber\(^{58}\) discovered in a family with hereditary cyanosis having a dominant transmission pattern. The visible-light-absorption spectrum of this methemoglobin differed from normal methemoglobin and it could be reproduced by the in vitro recombination of normal heme with the globin separated from the hemoglobin of the affected individuals.\(^{58}\) Gerald\(^{59}\) later showed that a similar abnormal methemoglobin discovered by him in Boston had a different electrophoretic mobility from that of methemoglobin A, if electrophoresis was performed at neutral pH.

Since then, individuals or families with an abnormal methemoglobin have been found in many parts of the world.\(^{60,66}\) The absorption spectra of several of these abnormal methemoglobins showed characteristic differences which suggested molecular heterogeneity despite their seemingly identical physiologic effect, the production of hereditary cyanosis. This heterogeneity has been found to apply to other physicochemical characteristics, such as the ability of the hemes of these abnormal methemoglobins to react promptly with certain
anions (ligands), e.g., cyanide, and to the absorption spectra of such methemoglobin ligands. The similarity of the absorption spectra of acid methemoglobin M_Boston²⁹ and methemoglobin M_Saskatoon⁶¹ to fluoride methemoglobin A suggested to Gerald and George⁵⁵,⁶¹ that in these abnormal methemoglobins a second bond of the iron atom with the globin moiety is present in addition to the normal covalent link with the proximal histidine (fig. 1, 4). This second bond which apparently prevents the iron from combining reversibly with oxygen occurs between the sixth coordination position of iron and a reactive group in the polypeptide chain. This reactive side chain is thought to function in a manner analogous to the anions $F^-$, $CN^-$, $N_3^-$ which form complexes with normal methemoglobin.

This “internal complex” hypothesis has been strengthened by subsequent studies of the amino-acid sequences in four of the variants of Hb M, Hb M_Boston²⁹ ($\alpha_2\beta_2$), Hb M_Saskatoon ($\alpha_2\beta_2$), Hb M_Milwaukee 1 ($\alpha_2\beta_2$)³³ and Hb M_Watertown ($\alpha_2\beta_2$). The amino acid substitutions in these hemoglobin mutants have occurred in a position in steric proximity to the iron atom and the “internal ligand” is furnished by the reactive side chain of the substituting amino acid, e.g., the phenolic group of tyrosine in position 58 of the $\alpha$ chain in Hb M_Boston ($\alpha_2\beta_2$) (fig. 4) and in position 63 of the $\beta$ chain in Hgb M_Saskatoon ($\alpha_2\beta_2$). These positions are normally occupied by the distal histidine, in close steric proximity to the sixth coordination position of heme iron. Because of the helical arrangement of the polypeptide chains,
the amino acid in position 67 of the \( \beta \) chain, if endowed with a reactive group, is also capable of forming a bond with the heme iron (fig. 4). This appears to be the case in \( \text{Hb } \text{M}_{\text{Milwaukee}} \) (\( \alpha_{2}^{A} \beta_{2}^{67\text{Gln}} \)).\(^{35,60}\) Certain conceptual difficulties arise in the application of the internal complex hypothesis to \( \text{Hb M}_{\text{Iwate}} \) (\( \text{Hb } \text{M}_{\text{Kankakee}} \)).\(^{63,65,67}\) In this hemoglobin, tyrosine replaces histidine in position 87a,\(^{68,69}\) normally the site of the covalent bond with the fifth coordination position of the heme iron (fig. 1, 4). It seems unlikely that tyrosine in this position could both form a bond with the fifth and interfere with the reversible oxygenation of the sixth coordination position of iron. It is more likely that histidine 58 has assumed the function of forming the “normal” bond with heme iron, thus enabling tyrosine in position 87 to form a stable internal complex with ferric iron (fig. 4).

Under normal in vivo conditions, some oxidation of hemoglobin to met-hemoglobin occurs, but the reducing system of the normal erythrocyte keeps the concentration of methemoglobin at about 1 per cent. The enzyme system that reduces methemoglobin A functions normally in erythrocytes of individuals with \( \text{Hb M} \), but it is unable to reduce methemoglobin M, which, therefore, remains in the oxidized state. Its concentration, usually 25–30 per cent of the total hemoglobin, is limited only by the number of abnormal hemoglobin molecules present. The abnormality has been observed only in the heterozygous state, since homozygosity is probably not compatible with life.

The differences between hemoglobin \( \text{M}_{\text{Benton}} \) (\( \alpha_{2}^{58\text{Glu}} \beta_{2}^{A} \)) and of \( \text{M}_{\text{Saskatoon}} \) (\( \alpha_{2}^{A} \beta_{2}^{63\text{Trp}} \))\(^{8}\) are of great theoretical interest. In either hemoglobin the distal histidyl, the amino acid residue closest to the site of the iron-oxygen bond, is replaced by tyrosyl and one could therefore expect that the hemes of these two abnormal hemoglobins react in a similar manner with ligands and with oxygen. This, however, is not the case. They show marked differences in their absorption spectra, reactivity with ligands, reducibility with dithionite, and probably also oxygen affinity. The reaction of the \( \beta \) chain variant with cyanide,\(^{62,64}\) proceeds only slightly slower than that of \( \text{Hb A} \) and the resulting cyanmethemoglobin spectrum is normal,\(^{56,64}\) whereas \( \text{Hb M}_{\text{Benton}} \) reacts very slowly with cyanide and the spectrum of cyanmethemoglobin is markedly abnormal.\(^{59}\) In addition, the \( \alpha \)-chain variant has been found more resistant to reduction by dithionite than the \( \beta \)-chain variant.\(^{56}\) Thus, the heme groups of the \( \alpha \) - and \( \beta \) - chains react differently with their environment when their distal histidyl is replaced by tyrosyl. That this difference might also be present normally is suggested by the behavior of \( \text{Hb H} \),\(^{71}\) a tetramer of the \( \beta \)-chain (\( \beta_{4} \))\(^{72}\) occurring in association with \( \alpha \)-thalassemia,\(^{73}\) probably as a sequel to diminished \( \alpha \)-chain synthesis. \( \text{Hb H} \) is unstable, has a high oxygen affinity, lacks heme-heme-interaction, and fails to show the Bohr effect.\(^{74}\) These peculiarities of hemoglobins M and H and the previously mentioned studies of Muirhead and Perutz\(^{28}\) point to differences in the behavior of \( \alpha \) and \( \beta \) -chains during oxygenation. The distance between the \( \beta \)-chain-hemes decreases after oxygenation,\(^{28}\) but this narrowing has not been observed in the crystal structure.

\*Hemoglobin \( \text{M}_{\text{Chicago}} \)\(^{64}\) proved to have the same structural abnormality as that reported for \( \text{Hb M}_{\text{Saskatoon}} \).\(^{70}\)
of oxyhemoglobin H.\textsuperscript{75} It appears that the “empty space” between the two $\beta$ chains in the normal reduced hemoglobin molecule facilitates the reaction of the heme irons with oxygen. This in turn is associated with intramolecular conformational changes and a diminution of the inter-chain space. X-ray crystallographic study of hemoglobin suggests that this structural rearrangement of the molecule during oxygenation and deoxygenation depends on the presence of two pairs of unequal polypeptide chains. It is likely that a clear picture of the intramolecular processes occurring during gas exchange will evolve in the near future.

Hbzuricii\textsuperscript{34,35} is an interesting abnormality in which the distal histidine of the $\beta$-chain is replaced by arginine ($\alpha_2^\beta_2\text{Arg}$). This substitution apparently does not disturb the intramolecular balance significantly until the carrier ingests certain drugs, especially sulfonamides, primaquine, and other agents which also produce lysis of erythrocytes deficient in glucose-6-phosphate dehydrogenase. Under the influence of these oxidizing agents the hemoglobin molecule becomes unstable, large inclusion bodies appear in the erythrocytes, excessive amounts of methemoglobin form and severe hemolysis ensues. It is of interest that reticulocytosis has been found in patients with hemoglobin M\textsubscript{MIIwakakei},\textsuperscript{190} and a child with HbM\textsubscript{Kankakee} ($\alpha_2^\beta_2\text{Gly}$) has been found to have hemolytic anemia,\textsuperscript{64,70} for which no other cause could be detected. On the other hand, the five individuals with Hb Mia-at,\textsuperscript{19} observed in Illinois (Hb MKankakee) have no hemolytic process.\textsuperscript{65} It is possible that the presence of an anomaly in the $\beta$-chain of Hb M mutants leads to greater molecular instability than an analogous amino acid substitution in the $\alpha$-chain.

Another hereditary hemolytic anemia with apparently dominant transmission deserves consideration here, although the primary genetic abnormality probably differs from that of the hemoglobinopathies. This uncommon and severe hemolytic anemia\textsuperscript{76,77,78,79,80} is characterized by large, usually single, erythrocyte inclusion bodies and the urinary excretion of a pigment of probably dipyrrolic structure.\textsuperscript{75} The inclusions resemble Heinz bodies and appear to consist of denatured hemoglobin and ribonucleoprotein. Conventional electrophoresis of hemolystates of some patients revealed a slow moving hemoglobin amounting to approximately 10 per cent of the total hemoglobin. In the patient described by Shibata and his co-workers\textsuperscript{80} this amount varied between 7.5–14 per cent, suggesting molecular instability or varying production of this abnormal fraction (Hb Ube 1). The fingerprint of the tryptic digest of this abnormal fraction was normal. However, no reactive SH-groups were found by titration with p-chloro-mercuribenzoic acid and they were considered to be blocked by an unknown mechanism. These SH groups belong to the cysteinyl residues in position 93, adjacent to the proximal histidyls of the two $\beta$-chains.

This disease, therefore, cannot be labelled a hemoglobinopathy in the strict sense. The alteration of the electrical charge of part of the hemoglobin does not appear to be the result of a genetically determined amino acid substitution, but perhaps of an enzymatic abnormality which leads to spontaneous
denaturation of hemoglobin. Although the cause of the blocking of the titratable SH groups in this abnormality remains unknown, the severity of the hemolytic anemia is an indication of the physiologic importance of the cysteinyl residue in close proximity to the heme group. This cysteinyl residue has not yet been found to be the site of a mutational substitution in an abnormal hemoglobin. It is possible that such a genetically determined replacement of this amino acid also would be associated with a severe hemolytic anemia.

The molecular anomalies of hemoglobin discussed in this review are experiments of nature which we have begun to comprehend only during the last decade. This knowledge has enhanced our understanding of the function and structure of normal hemoglobin. We hope that in the future we might also learn how to modify the noxious effects of some of the abnormal hemoglobins by influencing their molecular structure.

**SUMMARIO IN INTERLINGUA**

Le presente revista del hemoglobinopathias se restringe al discussion de seligite materiales de importantia pro nostre comprension del bases molecular e submolecular de anormalitates de hemoglobina. Illo se concerne principalmente con le relation inter le anormal structura molecular de mutantes de hemoglobina e lor pathogenicitate.

Le revista coperi un serie de octanta titulos in le litteratura. Le conclusion general es formulate que le enumerate anormalitates de hemoglobina es experimentos del natura cuje comprension promove nostre comprension del function e del structura de hemoglobina normal e alimenta le spero que in le curso del tempore nos va apprender a modificar le nocive effectos de certe hemoglobinas anormal per modificar lor structura molecular.

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