Hemoglobin Gun Hill: An Unstable Protein Associated with Chronic Hemolysis

By Ronald F. Rieder and Thomas B. Bradley, Jr.

Most of the abnormal hemoglobins found in man are not associated with clinical disorders. A few hemoglobins, S, C, D_{Punjab} and E, produce hemolytic disease in the homozygous state or when associated in the heterozygous state with another abnormal hemoglobin or thalassemia. Individuals inheriting only one gene for these hemoglobins generally do not have shortened red cell life spans. In contrast, hemoglobin Zürich, hemoglobin Köln, hemoglobin Genova, hemoglobin Sydney, hemoglobin Hammersmith, and hemoglobin Sinai are unstable proteins which produce increased red cell destruction even when constituting less than 50 per cent of the total erythrocyte hemoglobin. Patients with relatively small amounts of hemoglobin H often exhibit evidence of hemolytic disease. In addition to these well-defined hemoglobins, several examples have been reported of chronic hemolysis in patients possessing unstable abnormal hemoglobin fractions which have not yet been completely characterized.

The present report is concerned with a new hemoglobin variant, hemoglobin Gun Hill. This hemoglobin was found in a Caucasian man with a long history of mild jaundice and in one of his daughters. Structural analyses have suggested that there is a deletion of five sequential amino acid residues in the \( \beta \) chains of hemoglobin Gun Hill and have demonstrated that the \( \beta \) chains lack heme groups. The molecule is unstable, and in the heterozygous state it is associated with chronic hemolysis. In vivo and in vitro studies suggest that hemoglobin Gun Hill is synthesized at a rate greater than that...
of hemoglobin A although present in the peripheral blood in amounts less than that of hemoglobin A. The data indicate a greatly increased turnover rate of the abnormal fraction.

**METHODS**

Standard methods were employed for the routine hematologic examinations. The concentrations of hemoglobin solutions were determined by comparing the optical densities at 540 nm of the cyanmethemoglobin derivatives to that of a reference standard (Acuglobin, Ortho Pharmaceutical Corp.), or by measurement of protein nitrogen by the Kjeldahl method. Because of the absence of half the heme groups in hemoglobin Gun Hill, the extinction coefficient at 540 nm of cyanmethemoglobin Gun Hill is half that of cyanmethemoglobin A.

Reduced glutathione was measured by the method of Beutler, Duron and Kelly, glucose-6-phosphate dehydrogenase activity by the method of Marks, pyruvate kinase by the method of Gross, Schroeder and Brownstein, and glutathione reductase (TPNH-dependent) by the method of Gross and associates.

Standard methods were employed for hemoglobin electrophoresis on filter paper, agar gel, starch block, starch gel. The procedure of Betke, Marti and Schlicht was used to assay alkali-resistant hemoglobin. The ferrohemoglobin solubility test was performed according to Itano.

Studies of the effects of redox dyes on red cells and hemoglobin solutions were performed as previously described.

In vitro studies of hemoglobin synthesis by reticulocytes utilizing leucine-14C and in vivo studies with 59Fe were carried out by methods previously detailed. Washed red cells containing 5 to 10 per cent reticulocytes were incubated at 37 C. in a modified Krebs-Henseleit solution containing glucose, amino acids, penicillin, and streptomycin. Leucine-14C with a specific activity of 240 mc./mM was added as the isotopic tracer. At varying intervals aliquots were removed and the red cells were lysed by freezing and thawing. The stromal material was removed by centrifugation, and the supernatant hemoglobin solution was freed of unincorporated radioactivity by dialysis or passage through a Sephadex G-25 column. Nonhemoglobin proteins were removed by Amberlite CG-50 column chromatography. Hemoglobin A and hemoglobin Gun Hill were separated by starch-block electrophoresis. Samples of hemoglobin Gun Hill prepared in this manner contained hemoglobin A2. In some instances, nonhemoglobin proteins were removed and hemoglobin A, A2, and Gun Hill separated by carboxymethyl cellulose chromatography. Both methods of preparation gave similar results. Radioactivity was measured in a Nuclear-Chicago low-background gas-flow counter. Sufficient background and sample counts were taken to assure a sample error of less than 5 per cent.

For the in vivo studies of hemoglobin synthesis, 10 μc. of 59Fe citrate were given intravenously. Blood samples were drawn two days later and periodically thereafter during the next three weeks. For each of the samples, the red cells were separated, washed, and lysed. Hemoglobin A and hemoglobin Gun Hill were separated by starch-block electrophoresis, and the radioactivity in each of the fractions was determined with a well-type, solid crystal, NaI scintillation counter.

**RESULTS**

**Clinical Studies**

R.M., the propositus, is a 41 year old man of German-English extraction, who was referred for study because of a suspected hematologic disorder. The patient has been aware of mild scleral icterus since age 13. Some slight increase in the intensity of the jaundice has been noted in association with upper respiratory infections, but there have been no episodes of deep jaundice, dark urine, or light stools. He has known of an enlarged spleen for several
HEMOGLOBIN GUN HILL

Table 1.—Hematologic Values of Family Members Heterozygous for Hemoglobin Gun Hill

<table>
<thead>
<tr>
<th>Test</th>
<th>R.M. (Father)</th>
<th>E.M. (Daughter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (Gm./100ml.)</td>
<td>13.5 (16.2)*</td>
<td>12.6 (15.1)*</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>48</td>
<td>45</td>
</tr>
<tr>
<td>Red blood cell count (×10⁶/mm.³)</td>
<td>5.68</td>
<td>4.44</td>
</tr>
<tr>
<td>Mean corpuscular volume (μ³)</td>
<td>85</td>
<td>101</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin (μg.)</td>
<td>24 (28)*</td>
<td>29 (34)*</td>
</tr>
<tr>
<td>Mean corpuscular hemoglobin concentration (%)</td>
<td>28 (34)*</td>
<td>28 (34)*</td>
</tr>
<tr>
<td>Reticulocytes (%)</td>
<td>4.6</td>
<td>7-10</td>
</tr>
<tr>
<td>Red blood cell survival (51Cr T ½ days)</td>
<td>17</td>
<td>—</td>
</tr>
<tr>
<td>Plasma ⁵⁹Fe clearance (T ½ min.)</td>
<td>37</td>
<td>—</td>
</tr>
<tr>
<td>⁵⁹Fe 90% reappearance time (days)</td>
<td>5.5-6</td>
<td>—</td>
</tr>
<tr>
<td>Fecal urobilinogen (Ehrlich units/day)</td>
<td>404</td>
<td>—</td>
</tr>
<tr>
<td>Ferrohemoglobin solubility (Gm./l)</td>
<td>4.3</td>
<td>4.3</td>
</tr>
<tr>
<td>Alkali-resistant hemoglobin (%)</td>
<td>2.4</td>
<td>2.4</td>
</tr>
<tr>
<td>Hemoglobin Gun Hill (%)†</td>
<td>21 (30)*</td>
<td>20 (29)*</td>
</tr>
</tbody>
</table>

*The figures in parenthesis are the values obtained when correction is made for the altered extinction coefficient of hemoglobin Gun Hill at 540 μm.
†Based on estimates from starch-block electrophoresis assuming a value of 3% for the percentage of hemoglobin A₂.

years. There is no history of drug reactions or fatty food intolerance. The patient's father was thought to have had mild scleral icterus but was unavailable for study. There is no other family history of jaundice or blood disease. Physical examination revealed a well-developed man with yellow-tinged scleras. The spleen was enlarged to 3 cm. below the left costal margin. No other physical abnormalities were noted.

The results of the routine hematologic studies are listed in Table 1. The white blood cell count was 4,700 per cu.mm. and the platelet count ranged from 79,000 to 139,000/cu.mm. The erythrocytes were normochromic with slight anisocytosis and poikilocytosis. No inclusion bodies were noted in blood films stained with Wright-Giemsa or methyl violet.

Although the MCHC and MCH indicated that the red cells were hypochromic (Table 1), the peripheral smear appeared to be normochromic. This discrepancy probably reflects the deficiency of heme groups in hemoglobin Gun Hill. The red cell indices are related to the optical absorption of the heme groups, whereas the staining of the peripheral smear depends upon the protein content of the red cells. When corrected for the anomalous heme absorption, the red cell indices were within normal limits.

The serum bilirubin was 2.7 mg./100 ml. (direct 0.1 mg.). Liver-function tests gave normal results. No bile was found in the urine, and urobilinogen was detected in a 1:4 dilution. The serum haptoglobin level was 24 mg. per cent.

The presence of an elevated reticulocyte count, increased indirect-reacting bilirubin, and a normal hematocrit value (Table 1) indicated compensated hemolysis. Accelerated peripheral red blood cell destruction was demonstrated
by a shortened $^{51}$Cr-tagged erythrocyte half-life of seventeen days. Studies of ferrokinetics showed that iron was cleared abnormally rapidly from the plasma and had an early reappearance peak. Fecal urobilinogen excretion was increased. The erythrocyte osmotic fragility was normal.

The concentration of methemoglobin in the whole blood was normal. Assay of erythrocyte glucose-6-phosphate dehydrogenase, pyruvate kinase, and TPNH-dependent glutathione reductase gave normal or increased values consistent with a young red cell population. The level of reduced glutathione was slightly decreased. These studies were performed by Dr. Ernst R. Jaffé.

Findings suggesting hemolytic disease were also evident in the blood of E.M. (Table 1). She is 10 years old and is the only one of the three daughters of R.M. to possess the abnormal hemoglobin. E.M. has always been in good health and jaundice has never been observed. Physical examination revealed no scleral icterus, and the spleen was not palpable. The serum bilirubin was 2.0 mg./100 ml. (direct 0.3 mg.).

Both patients had slightly increased levels of alkali-resistant hemoglobin (Table 1).

Characterization of the Abnormal Hemoglobin

Electrophoresis of hemolysates prepared from the blood of R.M. and E.M. revealed an abnormal hemoglobin. On filter paper at pH 8.6, hemoglobin Gun Hill migrated with a mobility less than that of hemoglobin A and approximately that of hemoglobin S (Fig. 1). On the unstained paper, there appeared
Fig. 2.—Starch-gel electrophoresis at pH 8.6 of hemoglobins Gun Hill, A, and S. The major, slow-moving (a) and minor, fast-moving (b) fractions of hemoglobin Gun Hill are seen in the hemolysate of the propositus (R.M.). Amido black stain. The anode is to the right of the figure. CAB indicates carbonic anhydrase B.

to be only a small amount of the abnormal fraction. On starch-gel electrophoresis at pH 8.6, the new hemoglobin was found in the position of hemoglobin A₂ and hemoglobin C (Fig. 2). A second minor abnormal component was noted in a position just anodal to the major hemoglobin Gun Hill band (Fig. 2). The appearance of the electrophoretic patterns reflected the deficiency of the heme groups in hemoglobin Gun Hill: the patterns when stained for protein revealed a relatively more prominent abnormal band than did the unstained or benzidine-stained preparations. On agar-gel electrophoresis at pH 6.2, hemoglobin Gun Hill did not separate from hemoglobin A.

The proportion of hemoglobin Gun Hill in hemolysates was determined by starch-block electrophoresis. Both the propositus and his daughter were found to have approximately 30 per cent abnormal hemoglobin (Table 1).

Thermolability of Hemoglobin Gun Hill. The heat stability of the abnormal hemoglobin was tested by the method of Dacie and associates. Cleared hemolysates containing hemoglobin Gun Hill became cloudy within 5 min.
Fig. 3.—Effect of brilliant cresyl blue on red cells containing hemoglobin Gun Hill. Incubation at 37 C. for 1 hr. (A), 3 hr. (B), 24 hr. (C). (D) shows the absence of inclusion bodies in normal red cells after 22 hr. incubation. The one reticulocyte in each frame is readily distinguished from the inclusion body–containing red cells.

after heating to 50 C. A definite precipitate was present after 15 min. and a flocculent precipitate was noted after 60 min. incubation. Similar results were obtained with solutions of purified hemoglobin Gun Hill. Normal hemolysates or solutions of purified hemoglobin A remained clear for more than 30 min. after heating to 50 C. and showed only a trace precipitate after one hour of incubation.

Effect of Redox Dyes on Hemoglobin Gun Hill. Red cells from patients R.M. were incubated at 37 C., with a 1 per cent solution of the supravital stain brilliant cresyl blue. This dye is a redox reagent which has been shown to produce Heinz-like inclusion bodies in red cells containing certain other unstable hemoglobins.4,37 Within one hour after exposure to the dye, multiple dark-staining inclusion bodies appeared in red cells containing hemoglobin Gun Hill. With longer incubation, the inclusion bodies increased in number, size, and intensity of staining (Fig. 3). Only occasional inclusions were noted in normal cells exposed to the dye for 24 hr. Similar results were produced with another redox dye, new methylene blue. No inclusions were noted after
incubation with the phenylmethane dye, crystal violet. This dye is not a redox reagent but can stain preformed Heinz bodies.²

When hemolysates containing hemoglobin Gun Hill or solutions of purified hemoglobin Gun Hill were exposed to redox dyes, there was rapid formation of amorphous precipitates. Hemoglobin A solutions developed precipitates less rapidly.

**Urinary Dipyrrol Excretion.** Dark urine was not excreted by the patients with hemoglobin Gun Hill. Nevertheless, an attempt was made to demonstrate increased amounts of urinary dipyrrol.³⁸,³⁹ Urine from patient R.M. was acidified and extracted with petroleum ether. The aqueous phase was heated to 80 °C and allowed to stand overnight at room temperature. It was then extracted with acidified butanol and the organic phase collected and evaporated to dryness under reduced pressure. A small amount of brown residue was present but was no greater than that obtained from a similarly treated equal volume of normal urine. When the butanol extract was treated with alcoholic zinc acetate, the precipitated material appeared to be similar in amount to that obtained from urine of a normal individual. It was concluded that the
presence of hemoglobin Gun Hill is not associated with increased dipyrrol excretion.

Synthesis of Hemoglobin A and Hemoglobin Gun Hill by Reticulocytes in Vitro

Red cells from patient R.M. containing 5 to 10 per cent reticulocytes were incubated with leucine-$^{14}$C, and the incorporation of radioactivity into hemoglobin A and hemoglobin Gun Hill was measured. The results of a representative experiment are shown in Figure 4. Incorporation was linear for one hour, and at 30, 60, and 240 min. of incubation, the ratio of the specific activities of hemoglobin Gun Hill and hemoglobin A was greater than 4:1.

From the observed specific activities and the known relative proportions of the two hemoglobins present, it can be calculated that the total radioactivity incorporated into hemoglobin Gun Hill and hence the net synthesis of that fraction was almost twice that of hemoglobin A.

At 240 min. of incubation, the specific activity of hemoglobin Gun Hill was 453 counts per minute per milligram of protein. The specific activity of hemoglobin A was 110 counts per minute per milligram of protein. The ratio of the amount of hemoglobin Gun Hill to hemoglobin A found in the blood was 1:2.3. Therefore, the ratio of the amount of radioactivity incorporated into hemoglobin Gun Hill to that incorporated into hemoglobin A was $453 \times 1:110 \times 2.3$ or 1.8:1.
Incorporation of Radioactive Iron into Hemoglobin A and Hemoglobin Gun Hill in Vivo

Patient R.M. was given 10 μc. of radioiron intravenously. The specific activities of the two hemoglobin fractions were determined two days after injection and intermittently thereafter during the following three weeks. The results are shown in Figure 5. Specific activities are given in terms of heme absorption at 540 μm to compensate for the altered heme and iron content of hemoglobin Gun Hill. For a period of one week after the administration of isotope, the specific activity of hemoglobin Gun Hill was greater than that of hemoglobin A. By day 12, the specific activities of the two fractions were approximately equal. Two days after injection, the specific activity of hemoglobin A was 1.8 counts per minute per optical density unit. The specific activity of hemoglobin Gun Hill was 4.0 counts per minute per optical density unit. The ratio of the amounts of hemoglobin A heme to hemoglobin Gun Hill heme in the blood was approximately 4:1. Therefore, the ratio of the total amount of radioactivity incorporated into hemoglobin A heme to that incorporated into hemoglobin Gun Hill heme was $1.8 \times 4:4.0$ or 1.8:1. Since molecules of hemoglobin A contain twice as much heme as molecules of hemoglobin Gun Hill, approximately equal amounts of newly synthesized protein of each type were present in the peripheral blood on day 2.

Discussion

The majority of mutant hemoglobins differ from hemoglobin A by a single amino acid substitution.\footnote{Two exceptions are hemoglobin C Harlem,\cite{ref1} in which there are two altered residues, and hemoglobin Freiburg,\cite{ref2} which has a deletion of a single amino acid. The various hemoglobins Lepore have a $\beta$-$\delta$ fusion chain in place of the normal $\beta$ chain.\cite{ref3} Hemoglobin H is composed of four $\beta$ chains\cite{ref4} and hemoglobin Bart's is a $\gamma$-chain tetramer.\cite{ref5} The deletion of five amino acids in the $\beta$ chain and the absence of one half of the normal heme complement\cite{ref6} make hemoglobin Gun Hill one of the most radically altered of the mutant forms of human hemoglobin.}

In spite of the bizarre nature of the molecule, the clinical consequences of possessing hemoglobin Gun Hill are relatively minor. A well-compensated, mild hemolytic process was found in the two patients, but has not caused any disability. One patient has splenomegaly and slight jaundice. The increased rate of red cell destruction stems most likely from the increased tendency of hemoglobin Gun Hill to undergo denaturation. A number of other examples are known of the association of hemolytic anemia with an unstable hemoglobin.\footnote{These disorders comprise one of the categories of hereditary Heinz-body anemia.\cite{ref7} In many cases, the clinical manifestations have included chronic hemolysis, erythrocyte inclusion bodies (Heinz-Ehrlich bodies), and a dark pigment in the urine. There have been several examples of congenital Heinz-body anemia, closely resembling the above cases, where no unusual or unstable hemoglobin has been detected.\cite{ref8}-\cite{ref12} Some of these latter cases may represent examples of variant hemoglobins, having the same net charge as hemoglobin A, which are not revealed by electrophoresis.}
In these disorders, large numbers of spontaneously formed Heinz bodies have been found only in the peripheral blood of individuals who have undergone splenectomy. Several examples have been recorded of the first appearance of large numbers of Heinz bodies subsequent to removal of the spleen.\textsuperscript{5,10,16,17,19,50,51} Crosby\textsuperscript{52} has suggested that the intact spleen is able to pluck such inclusion bodies from erythrocytes as they pass through the organ, without destruction of the red cells.

Neither of the patients with hemoglobin Gun Hill has undergone splenectomy, and they do not demonstrate circulating Heinz bodies. However, their red cells do have an increased tendency to form Heinz-like bodies when incubated with a redox dye. Similar inclusions developed when red cells containing hemoglobin H\textsuperscript{7} and hemoglobin Zurich\textsuperscript{4} were exposed to brilliant cresyl blue. Cells from some of the patients with congenital Heinz-body anemia have been shown to form inclusion bodies very rapidly when incubated with acetylphenylhydrazine.\textsuperscript{6,8,14,20,46,50} In some reports, inclusion bodies formed simply after incubation of whole blood at 37°C.\textsuperscript{8,53,54} Patients with hemoglobin Zurich developed erythrocyte inclusion bodies in vivo after ingestion of certain oxidant drugs\textsuperscript{3}.

The exact nature of Heinz-Ehrlich bodies is not known, but they probably represent precipitated hemoglobin.\textsuperscript{47,55,56,57} The dyes brilliant cresyl blue and new methylene blue which produced inclusions in red cells containing hemoglobin Gun Hill also rapidly caused precipitation of solutions of the hemoglobin. Similar results have been reported for hemoglobin Zurich.\textsuperscript{4}

The labile nature of hemoglobin Gun Hill was also illustrated by its precipitation upon heating to 50°C. Increased thermolability has been reported for several other unstable hemoglobins.\textsuperscript{8,10,16,18,53,54,58}

Many of the cases of congenital Heinz-body anemia have demonstrated markedly increased urinary pigment excretion.\textsuperscript{5,6,10,14,16,17,19,44-50,54} This material has been classified as a dipyrrylmethene compound\textsuperscript{38,39} in every case where it has been examined. The origin of this pigment is not known. Dark urine was not found in the patients with hemoglobin Gun Hill, and no increased pigment residue was detected upon extraction of the urine with butanol. The absence of increased dipyrroluria may be due, in part, to the deficient heme content of the Gun Hill molecule.

Unequal turnover of hemoglobin A and hemoglobin Gun Hill was indicated by studies of hemoglobin synthesis. Although the amount of the variant hemoglobin in the peripheral blood was only half that of hemoglobin A, reticulocytes in vitro incorporated more radioactive amino acid into hemoglobin Gun Hill than into hemoglobin A. The in vivo studies indicated that there were equal amounts of newly synthesized hemoglobin in the two fractions two days after injection of \textsuperscript{59}Fe. These data suggest that the 2:1 ratio of hemoglobin A to hemoglobin Gun Hill in the peripheral blood is a result of preferential destruction of hemoglobin Gun Hill. Such loss could result from removal of denatured hemoglobin Gun Hill from circulating red cells or preferential destruction of those cells containing the largest amount of hemoglobin Gun Hill.
of unequal turnover of heterogeneous hemoglobins has been obtained in studies on other patients with unstable fractions.4,10,16,19

Net synthesis of hemoglobin Gun Hill by reticulocytes, in vitro, was twice that of hemoglobin A. More rapid synthesis of the mutant molecule could be a result of the absence of heme groups on the β-chains. Winslow and Ingram have suggested that there is a delay in the synthesis of the hemoglobin polypeptide chains near the position where heme is attached. Absence of this slow point in the synthesis of hemoglobin Gun Hill β chains might result in more rapid synthesis of the mutant molecule.

Reticulocytes, however, are responsible for only the last vestiges of hemoglobin synthesis, and heterogeneous proteins may have different rates of decline of synthesis as the erythrocyte matures.61 Synthetic ratios in reticulocytes may not reflect the ratios in bone marrow cells where the bulk of the hemoglobin is manufactured.

The lability of hemoglobin Gun Hill is not surprising in view of the marked structural alteration of an area of the β chain which is intimately involved with the binding of the heme moiety. This region, the corner between the F and G helices, is adjacent to the histidine residue in position 92, which normally forms a coordinate bond with the iron in the heme group.62 The structural alterations in the unstable hemoglobins Köln and Ube-15 occur in this same region. In hemoglobin Zürich, the mutation involves the distal histidine in position 63,68 which also forms a bond with the heme group. In hemoglobins Sydney and Hammersmith, other residues which stabilize the heme group are affected.

The mechanism by which the presence of an unstable hemoglobin results in hemolysis is not known. It has been postulated that Heinz bodies may attach to the red cell membrane by means of a mixed disulfide bond.64 If so, this might lead to changes in membrane function resulting in hemolysis. An alteration in the viscous properties of erythrocytes containing Heinz bodies might also affect red cell life span.65

**Summary**

A new unstable hemoglobin variant, hemoglobin Gun Hill, is reported. Two members of a Caucasian family were found to be heterozygous for the abnormal hemoglobin. Evidence of mild chronic hemolysis was found in both individuals.

Heinz-Ehrlich bodies were absent from the peripheral blood of the patients with hemoglobin Gun Hill, but Heinz-like inclusions were readily produced in their red cells upon in vitro incubation with the redox dyes brilliant cresyl blue or new methylene blue. Precipitation of hemoglobin occurred when solutions of hemoglobin Gun Hill were exposed to the dyes or were heated to 50 C. Previous studies have shown that there is a deletion of five amino acids in the β chains of hemoglobin Gun Hill. This results in impaired heme binding, and the abnormal molecule lacks half the expected number of heme groups. These structural alterations are probably responsible for the instability of the abnormal hemoglobin.

Studies of the relative rates of synthesis of hemoglobins A and Gun Hill,
using in vitro and in vivo technics, indicated an increased turnover rate for the variant protein. The evidence also suggested that hemoglobin Gun Hill is synthesized more rapidly than hemoglobin A.

SUMMARIO IN INTERLINGUA

Es reportate un nove instabile variante hemoglobinic, hemoglobina Gun Hill. Duo membros de un familia de racia blanca esseva recognoscite como heterozygoticos pro le hemoglobina anormal. Esseva trovate in ambe individuos evidentia pro un leve hemolyse chronic.

Corpores Heinz-Ehrlich esseva absent ab le sanguine peripheric del patientes con hemoglobina Gun Hill, sed inclusiones Heinz-oide esseva facile a producer in lor erythrocytos post incubation in vitro con le colorantes redox brilliante blau cresylic e nove blau methylenic. Le precipitation de hemoglobina occurreva quando solutiones de hemoglobina Gun Hill esseva exponite al colorantes mentionate o quando illos esseva calefacite usque ad 50 C. Previe studios ha monstrate que cinque amino-acidos es supprimite in le catenas (3 de hemoglobina Gun Hill. Isto resulta in un defective ligation de heme, e le molecula anormal es sin un medietate del expectate gruppos hemic. Il es probable que iste alterationes structural es responsabile pro le instabilitate del hemoglobina anormal.

Studios del relative productivitate del synthesize de hemoglobina A e de illo del synthesize de hemoglobina Gun Hill—con le uso de technicas in vitro e in vivo—ha indicate un accelerate metabolismo pro le proteina aberrante. Le evidencia etiam suggestiona que hemoglobina Gun Hill es synthetisate plus rapidemente que hemoglobina A.

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