Hemoglobin Zürich.
II. Physicochemical Properties of the Abnormal Hemoglobin

By Fedor Bachmann and Hans R. Marti

Hitzig et al.¹ and Frick et al.² recently described two related patients who developed severe hemolytic anemia with erythrocytic inclusion bodies after the administration of sulfonamides. No enzyme abnormality could be found in the erythrocytes of these patients, but the starch block electrophoresis of the hemolysates revealed an abnormal hemoglobin. The mobility of the abnormal component at pH 8.6 was slightly faster than the mobility of hemoglobin S. Samples of this unusual hemoglobin were sent to Dr. Jonxis and Dr. Muller in Groningen, The Netherlands, and to Dr. Huisman in Augusta, Ga., who independently concluded that the abnormal hemoglobin was a hitherto undescribed variant.³,⁴ It was tentatively named Hgb Zürich. A clinical report together with studies on erythrocyte survival times is presented in a companion paper.² The present communication concerns the physicochemical properties of Hgb Zürich, enzyme activities in the patients’ erythrocytes and describes the conditions necessary for Heinz body production.

Materials and Methods

All investigations reported were carried out on samples of blood obtained from the original patient L. Sch., aged 28 years,¹ and from his uncle A. H., 63 years old. During our investigations, both subjects were in hematologically steady conditions. The blood samples, obtained by venipuncture, were collected either in ACD or ACDI (2.4 Gm. of Inosine per 100 ml. of ACD-solution) solutions. Hemolysates were prepared from erythrocytes which were washed three times with physiologic saline followed by the addition of two volumes of distilled water and 0.5 volume of carbon tetrachloride. After the hemolysate had stood for one hour or more at 4 C., the stroma was separated by centrifugation at 20,000 g for 30 minutes. 0.015 volumes of a 5 per cent potassium cyanide solution were added to the hemolysates with the exception of those examined by spectroscopy. Samples were stored at 4 C. or kept frozen at −20 C. until used.

A Beckman DU spectrophotometer was used for the spectral absorption curves and for the following determinations: Hemoglobin concentrations were determined by the cyanmethemoglobin method read at 540 μm; in chromatographic fractions, absorbancies were measured at 415 μm; methemoglobin was measured according to the method of Evelyn and Malloy.⁵

Filter paper electrophoresis was accomplished on the Spinco Durrum cell with 0.05 M barbital buffer at pH 8.6 or with 0.05 and 0.09 M phosphate buffer at pH 6.8 at 250 V for 14 hours. The samples were applied on slightly moistened and blotted Whatman 3 MM filter paper strips with a lambda pipette. Starch block electrophoresis was performed after the method of Kunkel and Wallenius, modified by Betke et al.⁶ Starch gel electrophoresis was performed after Smithies.⁷ Agar gel electrophoresis was done according to the method of Robinson et al.⁸ Moving boundary electrophoresis was carried out with a Perkin-Elmer.
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Electrophoresis Apparatus, Model 38 A. Buffers used included cacodylate 0.02 M-NaCl 0.08 M at pH 6.5 and pH 6.8, phosphate 0.02 M-NaCl 0.08 M at pH 7.4 and barbital 0.1 M at pH 8.6.

Cuvette chromatography was performed on Amberlite IRC-50 (chromatographic grade CG-50, 200–400 mesh; British Drug House), as described by Huisman and Prins.9 Carboxymethyl-cellulose (CMC) chromatography was carried out according to Huisman and Meyering10 in 45 cm. columns with an internal diameter of 1.0 cm. Carboxymethyl-cellulose was prepared in the laboratory from Whatman standard grade cellulose powder as described by Peterson and Sober.11 Hemoglobin was applied as cyanmethemoglobin unless otherwise stated. All electrophoretic and chromatographic operations were carried out in the cold room at 1–4 C.

The sickling test, solubility tests and the test for detection of Hgb H inclusion bodies by incubation with brilliant cresyl blue were performed according to Jonxis and Huisman.12 For the solubility test, 9.0 ml. of the indicated phosphate buffer were used instead of 8.0 ml. in order to obtain a final molarity of 2.24. After addition of 50 mg. hemoglobin, the volume was adjusted with distilled water to 10.0 ml. The alkali denaturation test was accomplished by the method of Betke.13 Heat stability tests were done on cyanmethemoglobin in thin glass tubes of 3 mm. diameter at 65 C. and 70 C. after a preincubation at 55 C. for 5 to 10 minutes. The heated samples were centrifuged at 20,000 g for 30 minutes. Extinctions of the clear supernatant were read at 540 mJ.

Methemoglobin formation by spontaneous oxidation in air was studied in whole blood, washed erythrocytes and in hemolysates at concentrations corresponding to 5 Gm. hemoglobin per cent. Dilutions were made in a diluent consisting of equal parts of phosphate buffer pH 6.6 and normal saline. Samples of 4.0 ml. each were incubated in 25-ml. Erlenmeyer flasks in a Dubnoff metabolic shaker at 37 C. At intervals, aliquots of 0.2 ml. were withdrawn for the determination of methemoglobin. The methemoglobin reduction test was performed according to Brewer et al.14 Glucose-6-phosphate dehydrogenase was assayed by a modification of Kornberg and Horecker’s method after Szeinberg et al.15 Glutathione stability tests and the experimental production of Heinz bodies in vitro with acetylphenylhydrazine were performed according to Beutler et al.16,17

Fingerprints: Concentration of eluate peaks from CMC chromatography was accomplished by adsorption and re-elution on small columns as described by Huisman and Meyering.18 Re-chromatography of these concentrates showed 90 per cent to 96 per cent of the hemoglobin in a single homogeneous peak. The dialyzed desalted concentrates were digested at pH 8.0 with trypsin. The soluble tryptic peptides were separated on Whatman 3 MM filter paper by a two-dimensional electrophoresis-chromatography technic as described by Ingram.18 Ninhydrin staining and other color reactions for the identification of histidine, arginine, sulfur-containing amino acids, tyrosine and tryptophane were carried out as described by Block, Durrum and Zweig.19

RESULTS

Electrophoretic Mobility

Filter paper electrophoresis of the patients’ whole hemolysates revealed a second component beside Hgb A, migrating between Hgb A and Hgb S (fig. 1). At pH 6.5 no separation was obtained, but the patients’ hemolysates seemed to move somewhat faster than Hgb A alone (not illustrated in fig. 1).

In agar gel electrophoresis at pH 6.5 no separation was obtained.

In free electrophoresis no separation of the cyanmethemoglobin forms of Hgb Zürich and Hgb A was obtained at pH 6.5 with a current of 14 mAmp./0.30 cm.2 cross-sectional area in a 2 ml. Tiselius cell over 250 minutes. The mobility of the single peak obtained was calculated as 2.4 x 10−5 cm.2/volt/sec.

On starch gel and starch block, a good separation of Hgb A and the ab-
normal component was accomplished at pH 8.6. The Hgb Zurich component had a slightly higher anodal mobility than Hgb S at pH 8.6. Elution of the separated Hgb A₁⁺₃, Hgb A₂ and Hgb Zurich fractions from the starch block on different examinations of L. Sch.'s and A. H.'s blood resulted in the following amounts (extreme values): Hgb A₁⁺₃ 61–77 per cent; Hgb A₂ 2.6–2.9 per cent and Hgb Zurich 20–36 per cent of total extractable hemoglobin. At pH 6.5 the mobility of Hgb Zurich was between Hgb A and Hgb S.

Chromatographic Behavior

On Amberlite IRC-50 cuvettes, good separation was obtained after prolonged chromatographic runs for 24 to 48 hours. If the relative mobility of Hgb A was arbitrarily assigned as 10, then the abnormal component had a mobility of 8–8.5 (fig. 2). Comparison with hemolysates of Hgb A/S and Hgb A/E revealed that the chromatographic migration of Hgb Zurich was faster than that of Hgb S, but slower than that of Hgb E. It was regularly observed that the abnormal component developed a brownish color during the chromatographic run, probably because of the formation of methemoglobin.

Quantitative isolation was obtained between the abnormal component and Hgb A using carboxymethyl-cellulose chromatography. Figure 3 (top section) illustrates representative data with Hgb A/S and (bottom section) a similar run with Hgb A/Zurich. The major chromatographic fractions from both hemolysates, corresponding to those identified as Hgb A₁ by starch block electrophoresis, were eluted at identical elution volumes, the pH being 7.49.
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The minor peaks eluted prior to the major chromatographic fractions at respective pHs of 7.35 and 7.39-7.41 correspond to the fast running Hgb A3 fraction in starch block electrophoresis at pH 8.6. The abnormal peaks in the two chromatograms were eluted under different circumstances. Both appeared following the major chromatographic peak, but Hgb Zürich appeared at a lower total elution volume and at pH 7.61, as compared to Hgb S which was eluted later and at pH 7.66. Further chromatographic runs with different elution gradients confirmed the finding that the Hgb Zürich peak was eluted earlier and at a lower pH than the Hgb S peak. In both chromatographies there was a very small peak between the main fraction of Hgb A (A1) and the main abnormal peak, designated as S' and ZH' respectively in figure 3. Alkali denaturation showed that the ZH' peak was alkali labile. Thus its properties were not those of Hgb F. Fingerprints of the isolated ZH' peak showed the pattern of Hgb Zürich (see below).

Although the chromatographic run was performed with highspun hemolysate in the cyanmethemoglobin form, there was always a very small layer of a brownish red fraction at the top of the column, which could not be eluted at pH 8.6. It probably consisted of denatured hemoglobin. This phenomenon was not observed with hemolysates containing hemoglobin A, F, S, E and C.

Other Physicochemical Properties

Absorption spectrography of the oxidized, the reduced, the methemoglobin, the cyanmethemoglobin and the carbonmonoxymethemoglobin form of the patient's hemolysate revealed no significant differences from Hgb A in the range from 270–700 μm.

Alkali denaturation. Hgb Zürich was denatured by alkali. Several determinations for alkali resistant hemoglobin in L. Sch. and A. H. always revealed
values below 0.67 per cent (normal range, 0.25–0.75 per cent) of total hemoglobin. However in the case of the two year old daughter of L. Sch., an elevated value for Hgb F has been reported with the alkali denaturation method (5.2 per cent) and in the CMC chromatography (5.4 per cent). 3, 20

Solubility test. No differences in solubility were observed between the reduced forms of Hgb A and Hgb Zurich in 2.24 M phosphate buffer pH 6.8.

Sickling test was negative.

Hemoglobin H—inclusion bodies test. The patients’ erythrocytes upon incubation with 1 per cent brilliant cresyl blue solution for two hours did not show inclusion bodies, as seen in hemoglobin H disease. 21

Experimental production of Heinz bodies. Erythrocytes containing Hgb Zurich, when incubated in vitro with acetylphenylhydrazine according to the method of Beutler et al., 16 showed definitely more marginal well-rounded Heinz bodies, 0.5–1 µ in diameter than normal erythrocytes (fig. 4). This behavior is observed in glucose-6-phosphate dehydrogenase deficient erythrocytes. However the experimentally produced inclusion bodies differed distinctly from those seen during L. Sch.’s acute hemolytic crisis after the ingestion of sulfa-
Fig. 4.—Experimental production of Heinz bodies by incubation of normal and Hgb Zürich-containing blood with acetylphenylhydrazine. Left: Normal. Right: Pat. L. Sch.

methoxypyridazine. The in vivo formed inclusion bodies were 2–3 μ in diameter, solitary, situated at the extreme margin of the red cell, and sometimes even disrupted the membrane. Both forms stained well with brilliant cresyl blue. The in vivo produced inclusion bodies stained also with Giemsa solution.2

The erythrocytes of L. Sch., when incubated with various concentrations of sulfamethoxypyridazine, did not develop an excess of Heinz bodies compared to normal erythrocytes. Either there was a marked formation of inclusion bodies in both normal and pathologic cells or there was only a very weak formation. Since it has been demonstrated that Cr51-labeled Hgb Zürich-containing erythrocytes are sensitive to a multitude of drugs, including primaquine, sulfamethoxypyridazine, sulfadimethoxine, sulfisomidine, sulfisoxazole and to the combined effect of sulfaproxyline and sulfamerazine,2 attempts were made to reproduce the Heinz body phenomenon by incubating washed Hgb Zürich-containing erythrocytes with compatible plasma of subjects treated with sulfamethoxypyridazine. Three normal persons were given 2 Gm. of sulfamethoxypyridazine daily for three days. On the fourth day blood was drawn from these subjects and our patient L. Sch. The washed erythrocytes of L. Sch. were mixed with the plasma of the normal subjects obtained before (kept frozen at −20 C. until used) and after the drug was administered. The mixtures were incubated at 37 C. and the production of inclusion bodies was followed for a period of 24 hours by an observer who was ignorant as to which samples contained the drug.
Fig. 5.—Heat lability tests with the cyanmethemoglobin form of a normal hemolyzate (N), the Hgb A1-fraction (A1) and the Hgb Zurich-fraction (Z) of patient L. Sch. at 65 C. and 70 C.

A significantly greater production of Heinz bodies was recorded in two of the three erythrocyte samples incubated with plasma obtained after sulfathmethoxypridazine treatment as compared with the aliquots incubated with plasma obtained before this drug had been given.

Heat lability. Hgb Zurich showed greater heat lability than Hgb A. Figure 5 illustrates heat denaturation experiments at 65 C. and 70 C. Hemolysates of normal blood and the Hgb A1 and Hgb Zurich fractions of our patient L. Sch., obtained by CMC chromatography, were examined in their cyanmethemoglobin form. The Hgb A1 fraction of our patient was not as stable as a normal un-fractionated hemolysate. This finding could have been due to minor contaminations with Hgb Zurich or to moderate denaturation during the chromatographic run. Hgb Zurich was significantly less stable than either the patient’s Hgb A1 fraction or the normal hemolysate. After 32 minutes of incubation at 65 C., the denaturation rate of Hgb Zurich was three times greater than that of the normal hemolysate and 1.9 times greater than that of the same patient’s Hgb A1 fraction. After eight minutes incubation at 70 C., the denaturation rate of the Hgb Zurich fraction was 2.5 times greater than that of the normal hemolysate and 1.9 times greater than that of the patient’s Hgb A1 fraction.

Methemoglobin formation and methemoglobin reduction. The methemoglobin content of L. Sch.’s blood during the hemolytic episode was 40 per cent but otherwise never exceeded 1 per cent in several examinations of freshly drawn blood. The rate of spontaneous methemoglobin formation was determined in the aqueous starch block eluates of patient L. Sch. After storage at 4 C. for four days, the Hgb A1 fraction showed 2.3 per cent methemoglobin; the Hgb Zurich fraction contained 3.5 per cent. This difference is not significant.

A further experiment was carried out by incubating washed erythrocytes
Fig. 6.—Methemoglobin formation at 37 C. with normal (N) and Hgb Zürich-containing blood (Z). A. Hemolysates; B. Washed erythrocytes.

and hemolysates of normal persons and those of patient A. H. in pH 6.6 phosphate buffer/normal saline at 37 C. in a Dubnoff metabolic shaker. It may be seen from figure 6 that the rate of methemoglobin formation by spontaneous oxidation in air is significantly accelerated in these hemolysates and erythrocytes containing Hgb Zürich. A similar phenomenon has been observed in cases with hemoglobin H disease and with hemoglobin M disease.

To decide whether this increased rate of methemoglobin formation by oxidation in air was due to a lability of the hemoglobin molecule or to a deficiency in an erythrocyte enzyme, numerous enzyme determinations were done.* All tests usually positive in the glucose-6-phosphate dehydrogenase deficient subjects were negative in both patients. The results listed in table 1 demonstrate that the reducing capacity of the patients' erythrocytes was normal. This is in contrast to the findings encountered in primaquine sensitive erythrocytes.

*Dr. H. D. Waller and Dr. G. W. Lohr (Marburg, Germany) performed extensive enzyme studies on the erythrocytes of L. Sch. and A. H. They found normal values for hexokinase, pyruvate kinase, phosphoglucone dehydrogenase and glutamic-pyruvic transaminase activity. Normal or slightly increased activities in A. H.’s, and slightly or moderately increased values in L. Sch.’s erythrocytes were observed for the following enzymes: Phosphoglucomutase, phosphoglucone isomerase, 6-phosphofructokinase, aldolase, triosephosphateisomerase, phosphoglyceraldehyde dehydrogenase, phosphoglyceratekinase, phosphoglyceromutase, enolase, lactic dehydrogenase, isocitric dehydrogenase, malic dehydrogenase, methemoglobin reductase (TPN-sensitive), glucose-6-phosphate dehydrogenase, glutathione reductase, adenylyl kinase, acid phosphatase, glutamic-oxalacetic transaminase. Oxygen uptake, pyruvate, lactate and total acid formation were normal in A. H.’s but slightly increased in L. Sch.’s erythrocytes. The red cell content of ATP, ADP, AMP, and DPN+ and TPN+ was normal in both patients.
Table 1.—Results of Glucose-6-Phosphate Dehydrogenase Activity, Glutathione Stability Test and Methemoglobin Reduction Test in Red Cells of L. Sch. and A. H.

<table>
<thead>
<tr>
<th>Test</th>
<th>L. Sch.</th>
<th>A. H.</th>
<th>Normal range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose-6-phosphate dehydrogenase activity</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Δ O.D., Gm. Hgb/min. elevated</td>
<td>elevated</td>
<td>32</td>
<td>15–28</td>
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<tr>
<td>Glutathione stability test</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>GSH mg./100 ml. red cells before incubation</td>
<td>71</td>
<td>67/93*</td>
<td>55–80</td>
</tr>
<tr>
<td>after incubation with APH</td>
<td>63</td>
<td>71/74*</td>
<td>50–80</td>
</tr>
<tr>
<td>Methemoglobin reduction test</td>
<td>4.5</td>
<td>10</td>
<td>&lt;10</td>
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</table>

*Samples taken on two different occasions.

Peptide Analysis

The chromatographically obtained fractions of Hgb Zurich and Hgb A (see Methods) were digested with trypsin. The soluble peptides were separated two-dimensionally by electrophoresis at pH 6.4, followed by ascending chromatography. Figure 7 shows fingerprint patterns of Hgb A and Hgb Zurich. The fingerprint of Hgb Zurich reveals three peptides staining well with ninhydrin (denoted by crosshatched spots in figure 7 and labeled A, B and C), not present in the Hgb A pattern. Peptide A stained well with the arginine reagent. None of the three 'new' peptides stained with the reagents for histidine, tyrosine, tryptophane or for sulfur-containing amino acids.

Since it has been shown that Hgb A, differs from Hgb A in the single respect that it contains glutathione, we examined the Hgb ZH' and the Hgb ZH (main peak) fractions (fig. 3) for differences in peptide—particularly glutathione—composition. Fingerprints of both peaks were identical except that an additional sulfur-containing peptide was detected in the ZH' peak. This peptide had a high anodal electrophoretic mobility at pH 6.4 and a chromatographic mobility intermediate between peptide 9 and 23; comparison with glutathione under identical conditions exhibited no differences.

Discussion

The discovery of an abnormal hemoglobin in the absence of any demonstrable erythrocytic enzyme deficiency suggests that the abnormal hemoglobin was itself the cause for the hemolytic episode recorded after the administration of sulfamethoxypyridazine to patient L. Sch. Hgb Zurich-containing erythrocytes behave like glucose-6-phosphate dehydrogenase-deficient red cells when incubated in vitro with acetylphenylhydrazine (APH). In contrast, the former do not show increased Heinz body formation as compared to normal cells when incubated in vitro with the hemolysis-producing drug sulfamethoxypyridazine, although it has been clearly demonstrated that in vivo their half-life is significantly shortened upon administration of a variety of drugs, including primaquine and sulfamethoxypyridazine. Similar findings were reported by Beutler et al. for primaquine-sensitive erythrocytes.
Fig. 7.—Fingerprints of the tryptic peptides of a Hgb Zurich-fraction from CMC-chromatography in comparison with normal Hgb A. The numbering refers to the original designation by Ingram. The three “new” Zurich-peptides are denoted by crosshatched spots.

Easily obtained a greatly enhanced production of Heinz bodies when incubating primaquine-sensitive erythrocytes with APH, but not with primaquine itself. Our in vitro finding that the plasma of normal subjects, to whom sulfamethoxypyridazine had been administered previously, induced more inclusion bodies in Hgb Zurich-containing erythrocytes than control plasma suggests that the drug acts indirectly upon the pathological cells.

Waller and Löhr confirmed our finding of a normal value for glucose-6-phosphate dehydrogenase in the two reported subjects; for a number of erythrocyte enzymes they even obtained slightly to moderately increased activities. An unusual finding was the detection of a significant activity of 1-phosphoglycerate dehydrogenase in L. Sch.’s erythrocytes, which they believe is absent from normal erythrocytes, but occasionally found in cases with Cooley’s anemia. The significance of this peculiar finding is not clear.

The finding with respect to the mechanism of Heinz body formation in patient L. Sch. appears to be unique. The cases of Schmid et al., Lange and Akeroyd and Scott et al., all with congenital hemolytic disease associated with red cell inclusion bodies, differ in many respects. Their patients had inclusion bodies in the absence of drug exposure associated with Heinz body anemia. In the case of Lange and Akeroyd, an instability of glutathione has been recorded and an increased amount of alkali resistant hemoglobin was found. In the case of Schmid et al., a probable elevation of the Hgb A₂ content was detected and in Scott’s case an abnormal hemoglobin was found with an increased alkali resistance. Its electrophoretic mobility at pH 8.6 distinguishes the latter from our abnormal hemoglobin in that it trails between Hgb A₁ and Hgb A₂, not separating distinctly from either of these fractions.
The physicochemical properties of Hgb Zürich resemble partly Hgb H and partly Hgb M. As in the case of Hgb H there is an increased tendency to form erythrocytic inclusion bodies and to form methemoglobin by spontaneous oxidation in air. Hgb H-containing hemolysates (in the form of oxyhemoglobin) form precipitates after storage at 4°C for several days. This phenomenon was also encountered in our chromatographic fractions of Hgb Zürich if the hemolysate was not converted into the cyanmethemoglobin form before introduction into the column and when the chromatographic run exceeded six days. Unlike Hgb H-containing erythrocytes, there was no formation of inclusion bodies upon incubation with brilliant cresyl blue. The heat lability was increased to a rate of two to three times greater than that of Hgb A. For Hgb H, a far greater heat lability is reported amounting to tenfold that of Hgb A.

With Hgb M of Kiese’s case, Hgb Zürich shares the property of an accelerated rate of methemoglobin formation by oxidation with air. This finding has been confirmed by Waller and Lohr. Muller and Kingma, to whom we sent blood samples of our patient L. Sch., investigated the significance of the three unusual peptides on the fingerprints of Hgb Zürich. Their work suggests that in Hgb Zürich an amino acid substitution occurs at the same position as in Hgb M

Analyzing the amino acid composition of the three "new" peptides in Hgb Zürich, they found two dipeptides, namely ala-arg and gly-lys, and a tripeptide, gly-lys-lys. When fingerprinting the isolated \( \beta \)-chains from Hgb Zürich, the three unusual spots were again present at the same place, but peptides \( \beta-20 \) and \( \beta-21^* \) were missing (since places 20 and 21 also contain a peptide from the \( \alpha \)-chain, the lack of peptides \( \beta-20 \) and \( \beta-21 \) is not demonstrated when whole Hgb Zürich is fingerprinted). Since it is known from the work of Braunitzer et al. that peptides \( \beta-20 \) and \( \beta-21 \) from normal hemoglobin differ in respect to one terminal lysine and consist of the amino acid sequence: ala-his-gly-lys-(lys), Muller and Kingma concluded that, in Hgb Zürich, histidine in place 63 was replaced by arginine. Consequently, tryptic digestion produces one additional cleavage product through action on the carboxyl group of arginine. The finding of three "new" peptides can therefore be explained by the substitution of one single amino acid and need not result from multiple defects as believed by Huisman et al.

In Hgb M

the same amino acid, his \( \beta^{63} \), seems to be replaced by tyrosine, resulting in significant changes of the spectrographic characteristics of the hemoglobin molecule. It is noteworthy that we could not find any significant changes in our spectrographic examinations of Hgb Zürich as compared to normal hemoglobin.

It is tempting to postulate that the replacement of the normally present histidine, probably closely related to the heme group of the \( \beta \)-chain, disturbs the normal interaction of heme and globin. This could explain the increased tendency of Hgb Zürich to form methemoglobin spontaneously.

\*\( \beta \)-Tp VII and \( \beta \)-Tp VII, VIII according to the new nomenclature proposed by Gerald and Ingram (J. Biol. Chem. 276:2155, 1961).
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From the studies of Jandl et al.\textsuperscript{36} and from Harley and Mauer,\textsuperscript{37,38} we know that methemoglobin formation always precedes the formation of inclusion bodies and is an essential stage in the destruction of hemoglobin to denatured products. When incubating acetylphenylhydrazine or phenylhydrazine with solutions of crystallized hemoglobin, Jandl et al. obtained granules with a striking similarity in size, shape, staining qualities and solubility characteristics to erythrocytic inclusion bodies. Fingerprints from tryptic digests of isolated inclusion bodies from Hgb Zürich-containing erythrocytes revealed the typical pattern of Hgb Zürich;\textsuperscript{36} this provides further evidence for the hemoglobin nature of the inclusion bodies in our patients.

Based on these findings, a hypothesis may be put forth that the change of one amino acid, probably near the heme group in the β-chain of Hgb Zürich, leads to an abnormal lability resulting in increased methemoglobin formation. Under normal conditions the reducing capacity of the patients’ erythrocyte dehydrogenases compensates for the increased methemoglobin formation. After ingestion of oxidizing agents, compensation fails. Further oxidation produces irreversible denaturation of the labile Hgb Zürich molecule.

SUMMARY

The physicochemical properties of Hgb Zürich were investigated. This new hemoglobin is characterized by an electrophoretic mobility between Hgb A and Hgb S at pH 8.6. Similarly it runs between Hgb A and Hgb S on amberlite IRC 50 and on carboxymethylcellulose chromatography. Fingerprints of the tryptic digests revealed three unusual peptides; their existence may be explained by the replacement of one histidyl group by an arginyl group in peptides 20, 21 of the β-chain. At 70°C, Hgb Zürich is destroyed three times faster than Hgb A. The erythrocytes and hemolysates of Hgb Zürich carriers show an increased tendency to form methemoglobin. When incubated with acetylphenylhydrazine, the erythrocytes behave like glucose-6-phosphate dehydrogenase-deficient red cells. Carriers of Hgb Zürich tend to develop severe hemolysis after the ingestion of primaquine and a variety of sulfonamides. Glucose-6-phosphate dehydrogenase activity, glutathione stability and methemoglobin reduction tests have been found normal.

SUMMARIO IN INTERLINGUA

Esseva investigate le proprietates de hemoglobino (Hgb) Zürich. Iste nove hemoglobina es characterisate per un mobilitate electrophoretic inter illos de Hgb A e Hgb S a pH 8,6. Similmente illo occupa un position inter Hgb A e Hgb S in chromatographia a Amberlite IRC-50 e in chromatographia a carboxymethylcellulosa. Studios del structura peptidic de digestos tryptic revelava tres peptidos inusual; lor existentia pote esser explicate a base del reimplacamento de un grupo histidyl per un grupo arginyl in le peptidos 20, 21 del catena beta. A 70°C, Hgb Zürich es destruite tres vices plus rapidente que Hgb A. Le erythrocytos e le hemolysatos de portatores de Hgb Zürich monstra un augmentate tendentia al formation de methemoglobina. Iste erythrocytos, quando illos es incubate con acetylphenylhydrazina, se com-
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... porta como erythrocytos deficiente in dishydrogenase de glucosa-6-phosphato. Portatores de Hgb Zürich ha le tendencia de disveloppar sever hemolyse post le ingestion de primaquina e varie sulfonamidos. Tests del activitate de dishydrogenase de glucosa-6-phosphato, del stabilitate de glutathiona, e del reductio de methemoglobina se ha monstrate normal.

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Hemoglobin Zürich. II. Physicochemical Properties of the Abnormal Hemoglobin

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