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Instability of Hemoglobin Molecule : Clinical and Laboratory Manifestations. —A Review. Part IV.

G. A. NIAZI and *Susumu SHIBATA

Department of Pathology, College of Medicine and Medical Sciences, King Faisal University, Dammama 31451, Saudi Arabia
**Kawasaki Medical School, Kurashiki 701-01, Japan*
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CLINICAL AND LABORATORY MANIFESTATIONS

The unstable hemoglobins are inherited as dominant characters and have only been observed in the heterozygotes. The homozygosity is probably not compatible with adult life and also because of low gene frequency for unstable hemoglobins, the homozygosity could be rare. However, sizable cases of unstable Hbs appear to have arisen because of spotaneous mutation in which both the parents being unaffected. This has been very well noticed in most of the cases with severe congenital Heinz body hemolytic anemia (CHBHA). These unstable hemoglobins are present usually in the range of 10–40% and the rest is all adult Hb, because the spleen selectively removes the Heinz bodies. The clinical picture of unstable hemoglobins is characterized by a variable degree of anemia and the clinical results of a given substitution or deletion, at least in part, can be correlated to its particular site in the molecule. A given mutant tend to produce similar clinical effects whether the mutant is present within the family or unrelated. The most suitable example will be that of Hb Köln. All the affected individual present a mild to well compensated hemolytic anemia specially after splenectomy. A severe CHBHA anemia has also been reported in the early childhood (e.g. Hb Bristol, Hb Hammersmith). There are several examples listed in Table 3 in which anemia is aggravated by viral or bacterial infection or following an induction of drug such as sulfonamides or acetaminophen e.g. Hb Hasharon, Hb Mequon, Hb Zürich etc. All the affected individuals had normal levels of hemoglobins and absent or minimal hemolysis unless they were exposed to oxidant stress. The anemia will be reverted on the withdrawal of drug. Sulfa drug exacerbated hemolysis has also been observed in case of Hb Torino, Hb Shepherds Bush, Hb Peterborough etc.

The presence of Heinz bodies has been reported in the case of severe hemolytic crisis but in certain cases it requires induction using appropriate conditions. Since staining procedures are simple and hematological findings are characteristic, test for Heinz bodies is extremely helpful in diagnosis and should invariably be used in conjunction with other routine hematological investigations in patients suspected for an unstable hemoglobin. Several patients give a history of passing dark urine which is due to dipyrrole pigment in the urine. However,

柴田 進 Reprint requests to : Susumu Shibata, M.D. Kawasaki Medical School
577 Matsushima, Kurashiki 701-01, Japan

TABLE 3. Clinical classification of unstable hemoglobin

(1) **Those that produce uncompensated severe hemolytic anemia :** Biba, Bicêtre, Bristol, Castilla, Hammersmith, Hirosaki, Indianapolis, Madrid, Mozhaisk, New Castle, Nottingham, Olmsted, Perth, Sabine, Savannah, Shuangfeng and Southampton
(2) **Those with moderate hemolytic disease compensated after splenectomy :** Ann Arbor, Böras, Christchurch, Coventry, Genova, Istanbul, Köln, Louisville, Mizuho, Niteroi, Santa Ana, St. Louis, Shepherds Bush, Torino, Volga, Wien and Yokohama
(3) **Produce mild hemolytic anemia except during crisis. In certain cases either due to infection or drug induction :** Altdorf, Altgeld Gardens, St. Antoine, Belfast, Buenos Aires, Burke, Fanin Lubbock, Freiburg, Guantanamo, Gun Hill, Hasharon, Hope, J-Calabri, Leslie, Leiden, Lufkin, Lyon, Mequon, Moabit, North Shore, Okaloosa, Petah Tikva, Peterborough, Philly, Port Phillip, Prato, Riverdale-Bronx, Rush, Saitama, Suan Dok, Seattle, Sydney, Tochigi, Tottori, Tübingen and Zürich
(4) **Presenting mild to moderate instability of hemoglobin molecule with no clinical manifestations :** Arya, Atlanta, Baylor, Bougardirey Mali, Brisbane, Bushwick, Camperdown, Caribbean, Cranston, Dakar, Djelfa, Etobicoke, Fort de France, Fort Worth, G-Ferrara, Grady, Henri Mondor, Hopkins-2, J-Rovigo, Khartoum, Manitoba, Miyashiro, Pasadena, Pontoise, Saki, Setif, Strasbourg, S-Travis, Sögn, Tacoma, Tours, Toyoake, Toulouse, Vaasa and Williamette

it should be noted that pigmenturia is neither a consistent finding in CHBHA nor related to the severity of hemolysis. There are several cases of chronic hemolytic anemia listed in Tables 1 and 2 in which pigmenturia is absent.

Reticulocytosis accompanied by raised levels of serum bilirubin and decreased haptoglobin is a usual finding in patient with CHBHA. The erythrocyte exhibits a minimal to marked aniso-and poikilocytosis. Hypochromia may be seen in some patients due to removal of precipitated globin by spleen and a reduction in the cellular concentration of hemoglobin. Leukocytes and the platelets are usually normal with the exception of thrombocytopenia in a few patients of Hb Köln. The measurement of cell life span by tagging red cells with Cr^{51} indicated a decreased survival. The red cell survival values (T/2) has been listed for many unstable variants in Tables 1 and 2, and some of them, such as Hb Hammersmith, Hb Southampton, Hb Mizuho etc., has exceptionally low red cell survival rate. The biosynthetic ratio has also been listed for many unstable hemoglobins, showing near normal to slightly inbalanced globin chain synthesis.

LABORATORY INVESTIGATIONS

All the unstable hemoglobins have an increased tendency to precipitate and the stability tests along with the test for Heinz bodies and hemoglobin electrophoresis are considered to be effective procedures for the detection of unstable hemoglobins.

Stability tests : Instability of hemoglobin molecule can be either determined by isopropanol precipitation or by thermal denaturation. The isopropanol method is simple and effective means of identifying the presence of an unstable hemoglobin. The unstable hemoglobin readily precipitates and almost quantitatively in this non-polar solvent as a result of reduction in binding forces within the

Hb molecule thus causing the precipitation. The unstable hemoglobins are also much heat labile and precipitate more easily as compared with the normal hemoglobins and stable hemoglobin variants. The heat stability test has therefore been widely used to demonstrate the presence of an unstable variant. This test can be performed at moderately elevated temperature (50°C) for one hour or at a higher temperature (60°C) for about 30 minutes. In either cases, the presence of unstable hemoglobin is indicated by turbidity. These stability tests have been fully described[179,180] and a normal control should be set up along with the sample under study and freshly drawn blood sample from a normal healthy subject should be used for this purpose.

Heinz bodies test : As said earlier that all the unstable hemoglobins has tendency to intracellular hemoglobin precipitation, being heat labile. These precipitates aggregate and form inclusion bodies (or Heinz bodies) which under

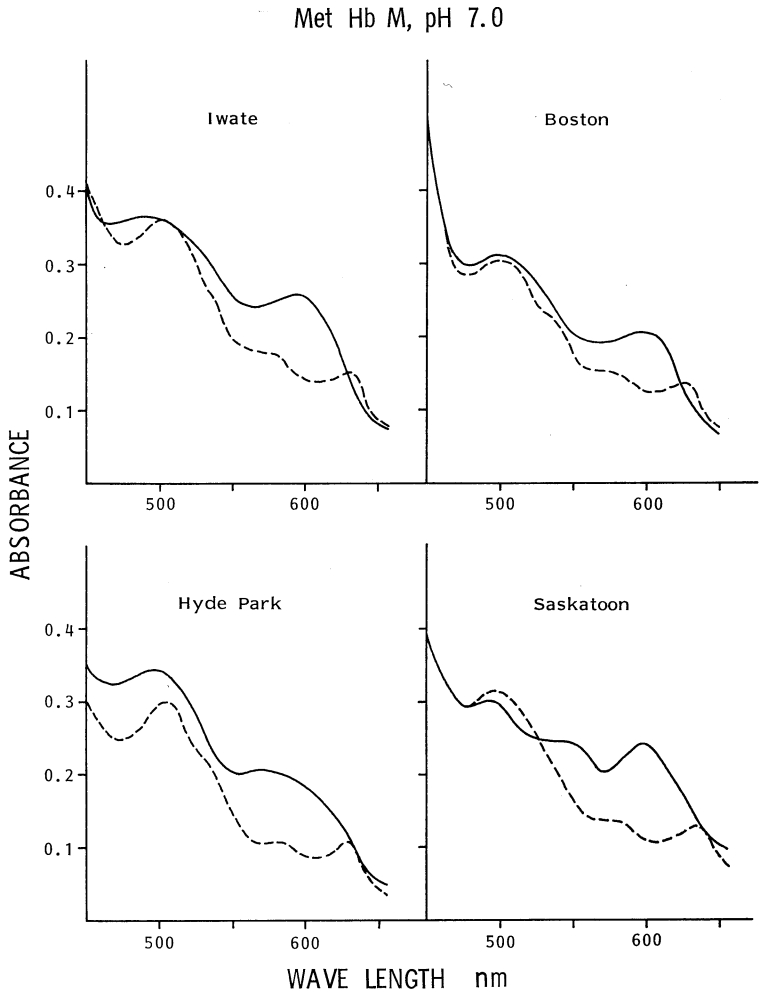

Fig. 4 Absorption spectrum of Hb Ms.

the appropriate staining conditions can be visualized in patients who have been splenectomized. The inclusion bodies are present in the large majority of red blood cells which can easily be seen wet unstained preparations or even better with brilliant cresyl blue staining. However in non-splenectomized patients formation of inclusion bodies has to be induced by incubation of sterile blood for 24 to 48 hours at 37°C, followed by staining with brilliant cresyl blue. However in many cases, the inclusions of unstable hemoglobins have been produced after incubation of blood of non-splenectomized patients with brilliant cresyl blue for a shorter period. The procedure[181] is very simple ; one part (1% solution of brilliant cresyl blue in a 0.9% NaCl) and two parts of blood are mixed and incubated at 37°C ; include at least one normal control with sample under investigation. Make dry blood films at 20 minutes, one hour and two hours intervals and examine under immersion oil. A convenient method for Heinz body staining was invented by Schwab and Lewis.[182]

Spectroscopy : Methemoglobins have characteristic spectral properties. It is unique among the hemoglobin derivatives they change their colour with change in pH. For this reason the acid methemoglobin (below pH 7.3) is brown and alkaline methemoglobin (above pH 7.4) is dark red. There is a well-known characteristic peak at 630 nm which is due to acid methemoglobin. The spectral properties of M-hemoglobins are different from those of normal methemoglobins[183] (Fig. 4) and thus provide a simple means of differentiation between Hb-M patients and patients with enzymopenic methemoglobinemia.

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