A Sensitive Cytochemical Staining Method for Glucose-6-Phosphate Dehydrogenase Activity in Individual Erythrocytes

I. Optimalization of the Staining Procedure

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Summary. A sensitive cytochemical staining method for glucose-6-phosphate dehydrogenase activity in individual human erythrocytes is described. This staining method can be used for the rapid routine discrimination of patients with a deficiency of the enzyme in its homozygote or heterozygote form, but also for quantitative localization of its activity in individual erythrocytes. The staining procedure in its optimal form consists of a treatment of the erythrocytes with sodium nitrite, then a "fixation" in 0.025% glutaraldehyde (under NADP+ protection of the active site of the enzyme), followed by incubation of the cells in suspension in the presence of tetranitro BT, 1-methoxyphenazine methosulphate and polyvinyl alcohol. Using this new technique, a sharp localization is obtained of the glucose-6-phosphate dehydrogenase activity, which enables discrimination between red cells with different levels of enzyme activity, as a consequence of enzyme deficiencies or age changes.

Introduction

Glucose-6-phosphate dehydrogenase (G6PDH) deficiency is the most common congenital enzyme deficiency in man, affecting more than 100 million individuals (World Health Organization 1967; Stuart et al. 1975). This hereditary abnormality is characterized by a diminished activity or stability of the enzyme (Beutler 1977). The gene for G6PDH is subject to many different mutations; at present, over 150 variants are documented (Beutler 1978, 1980). Some of these variants cause metabolic disorders by which the erythrocytes are most severely affected. With rare exceptions, the only clinical manifestation of G6PDH deficiency is haemolytic anemia, particularly after the administration of certain drugs, e.g. synthetic antimalarials like primaquine and pentaquine, but also sulfonamides like sulfapyridine and sulfanilamide, and other antibacterial agents (World Health Organization 1967; Beutler 1978). Haemolytic anemia may also occur during...
infections, in the neonatal period, under certain conditions of stress like diabetic acidosis or following ingestion of fava beans causing the well-known favism (Harris 1971; McKusick 1978).

The gene for G6PDH is carried on the X-chromosome and therefore full manifestation of the defective gene is found in the male hemizygote and the female homozygote. In the female heterozygote, however, a mixed population (mosaic) of normal and enzyme-deficient cells can be found (Beutler 1978). This is a result of random inactivation of one of both X-chromosomes (Lyon 1961; Beutler 1962; Beutler et al. 1962). The X-linked inheritance of the gene for G6PDH, together with the widespread occurrence of the enzyme deficiency in erythrocytes which can easily be investigated, has made this congenital disorder a favourite subject of study for biochemists, cell biologists and population geneticists. The red cell, as an object for study, is the more interesting in this connection, as this cell loses its ability to synthetize (enzyme) proteins, when it loses its nucleus in the late normoblast stage.

The discrimination between hemizygously deficient male patients or homozygously deficient female patients and nondeficient individuals is relatively simple on the basis of the biochemical determination of the G6PDH activity in haemolysates (Beutler 1971). On the other hand, at least about 20% of the heterozygotes cannot be detected on the basis of spectrophotometric assays on haemolysates (Fairbanks and Fernandez 1969). For the detection of heterozygotes, especially in family studies, but also for the diagnosis of homozygotes or hemizygotes when the majority of deficient cells has been eliminated by a haemolytic episode or diluted by blood transfusion, a cytochemical technique is a prerequisite, as an impression of the individual cells in the population should be gained (Stuart et al. 1975; Beutler 1978). Some other techniques are developed as well for this purpose, like the methaemoglobin elution test (Gall et al. 1965) and the ascorbate-cyanide test (Jacob and Jandl 1966; Deacon-Smith 1982) but all have disadvantages of being cumbersome and time-consuming, being unspecific for G6PDH deficiency and/or failing to give information about the individual cells (Stuart et al. 1975; Beutler 1978).

So, for this particular purpose Fairbanks and Lampe (1968) described a cytochemical assay for the estimation of G6PDH activity in individual erythrocytes. This technique was based on the reduction of the tetrazolium salt MTT into its formazan by NADPH2, formed by G6PDH activity, via the exogenous electron carrier Nile Blue sulphate and methaemoglobin. Several disadvantages in this method have been reported, however (Keller 1971; Gordon and Stuart 1974; Stuart et al. 1975). Cell-cell interaction may cause the formazan produced in active cells to precipitate on inactive cells. Under such conditions, the discrimination between normal and deficient cells becomes impossible. Numerous modifications of this MTT-method have been reported and also the use of many other tetrazolium salts has been tried (for a review see Stuart et al. 1975), but none of these attempts enabled the reliable and sensitive detection of G6PDH deficient heterozygotes.