

Beutler E. The glutathione instability of drug-sensitive red cells. A new method for the in vitro detection of drug-sensitivity. *J. Lab. Clin. Med.* **49**:84-94, 1957.

Normal, but not primaquine-sensitive red cells incubated for two hours with acetylphenylhydrazine in the presence of glucose, are able to maintain their level of reduced glutathione (GSH). Destruction of GSH in primaquine-sensitive cells occurs only in the presence of oxygen. [The *SCI*[®] indicates that this paper was cited 379 times in the period 1961-1977.]

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"Military service appeared inevitable to me as a second year medical resident at the University of Chicago in 1953. Since I was interested in hematology, the opportunity to work at the Army Malaria Research Project at the Stateville Penitentiary on primaquine-induced hemolytic anemia seemed ideal. The new antimalarial drug primaquine produced a severe hemolytic anemia in some black subjects. Yet when their red cells were examined by methods then available, they seemed to be entirely normal. I observed Heinz bodies in the red cells during the course of hemolytic crises, and was able to show that chemicals such as acetylphenylhydrazine produced a different pattern of Heinz body formation in vitro in primaquine-sensitive red cells than in normal red cells. Using inhibitors, I observed that normal cells treated with iodoacetate or arsenite behaved like primaquine-sensitive cells with respect to Heinz body formation. This turned my attention to red cell glutathione (GSH); the levels were lowered in primaquine-sensitive red cells, and moreover fell abruptly when primaquine was administered to sensitive subjects.

"Army life is not always predictable, and after one year's service at Stateville, I was transferred to Camp Detrick to serve out the remainder of my two-year term of active duty. When I returned to a junior faculty position at the University of Chicago in 1955, I attempted to unravel the biochemical basis of primaquine-sensitivity in red cells. It occurred to me that incubating blood from primaquine-sensitive donors with acetylphenylhydrazine might result in an abrupt fall in their GSH content. I proposed this project to a post-doctoral Fellow in the department, but he was not interested, and I undertook these studies myself.

"It was much more difficult to obtain blood from primaquine-sensitive subjects in civilian life than it had been in the prison. However, one of our subjects (a con-man) had been released from prison and volunteered to come to our clinic and donate blood for \$5. One day my donor happily told me that he had found a job but needed \$25 for new clothes. I advanced him the money for 5 donations; I have never seen him since. In spite of such difficulties, I was able to pursue these studies.

"After incubating blood from a primaquine-sensitive patient and a normal subject with acetylphenylhydrazine, I prepared a filtrate and added nitroprusside and cyanide. I can still remember my exhilaration (and almost disbelief) when, on my first attempt, no color developed in the filtrate from the incubated primaquine-sensitive sample. The 'GSH stability test' reported in the 1957 paper was the first reliable means for in vitro detection of primaquine-sensitivity. It quickly led to the discovery that the defect was sex-linked and that its basis was a deficiency in the enzyme glucose-6-phosphate dehydrogenase. Its principal effect was perhaps to produce awareness that the metabolism of red blood cells might be important in the origin of hemolytic disease."