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Beutler E, Duron O & Kelly B M. Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* **61**:882-8, 1963.
[Department of Medicine, City of Hope Medical Center, Duarte, CA]

A metaphosphoric acid-NaCl-EDTA filtrate of whole blood is reacted with a water soluble sulfhydryl reagent and the yellow color formed is measured at 412 nm. This technique provides a reliable method for measuring red cell reduced glutathione. [The *SCI*® indicates that this paper has been cited in over 780 publications.]

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The estimation of red cell glutathione (GSH) levels had played an important role in our laboratory since the discovery that red cells sensitive to the hemolytic effect of primaquine had unstable GSH.[1] We had modified a procedure originally described by Grunert and Phillips employing the nitroprusside reaction for glutathione.[2] While reasonably satisfactory, this method had serious drawbacks. Color formation was time-sensitive and temperature-sensitive, and it was necessary to assay a concurrent standard throughout the course of the day each time a sample was examined.

These deficiencies in our method of determining glutathione levels were brought into sharp focus in 1960 when, stimulated by the work of my colleague, Susumu Ohno, at the City of Hope, I conceived the idea that one of the two X chromosomes was genetically inactive. I reasoned that heterozygotes with glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, an X-linked trait, would have two populations of red cells—deficient cells and normal cells. Failing in my efforts to develop a histochemical method to estimate individual red cell G-6-PD activity, it occurred to me that the kinetics of GSH loss under oxidative stress could be very informative. It would be different in a suspension of cells each of which was one-half deficient in G-6-PD when compared with one in which one-half were normal and one-half were deficient. The necessity of carrying out frequent GSH determinations very accurately made the weaknesses of the technique we had been using, once merely annoying, a real obstacle.

Peter Jocelyn of the University of Edinburgh was spending a year in my laboratory as a visiting scientist. It was he who called my attention to the recent development by Ellman of a soluble disulfide reagent, 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB), which could be used to good advantage in performing GSH determinations.[3] Adaptation of this reagent to the measurement of red cell GSH was a rather straightforward task, and the method that we published was sufficiently robust that today, nearly 25 years later, we still use the same technique daily. It is presented essentially without alteration from the original in the third edition of our book *Red Cell Metabolism: A Manual of Biochemical Methods*.[4]

The importance of GSH in biology together with the ease with which this method can be implemented to provide accurate measurement presumably explains its wide acceptance. One of the great strengths of our laboratory has always been a hardworking, dedicated technical staff. Olga Duron and Barbara Mikus-Kelly helped greatly in developing this method.

1. **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. [See also: **Beutler E.** Citation Classic. *Current Contents* (28):18. 10 July 1978.]
2. **Grunert R R & Phillips P H.** A modification of the nitroprusside method of analysis for glutathione. *Arch. Biochem.* **30**:217-25. 1951. (Cited 600 times since 1955.)
3. **Ellman G L.** A colorimetric method for determining low concentrations of mercaptans. *Arch. Biochem. Biophys.* **74**:443-50. 1958. (Cited 300 times.)
4. **Beutler E.** *Red cell metabolism: a manual of biochemical methods.* Orlando, FL: Grune & Stratton, 1984. 188 p.

