

This Week's Citation Classic®

Paglia D E & Valentine W N. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 70:158-69, 1967.

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This paper described a direct spectrophotometric assay for measuring blood-cell glutathione peroxidase activity and presented some of the biochemical characteristics of that enzyme in human hemolysates. [The SCI® indicates that this paper has been cited in over 935 publications.]

Erythroenzymopathies: 1960s in Retrospect

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Most will recall the decade of the 1960s as a period of sociopolitical ferment, but it was no less so in our rather isolated and arcane world of erythrocyte metabolism. I had joined Bill Valentine's group in late 1964 as a postdoctoral research fellow after completing my residency in anatomic pathology at the University of California, Los Angeles. He and his colleagues, Kouichi R. (Corky) Tanaka and Shiro Miwa, had already established pyruvate kinase deficiency as the paradigm for anemias secondary to genetically induced defects of anaerobic glycolysis.¹ In quick succession, recognition of deficiencies of triosephosphate isomerase, hexokinase and glucosephosphate isomerase followed, and it became clear that a heterogeneous group of enzymopathies would likely emerge from those disorders traditionally grouped as "congenital nonspherocytic hemolytic anemias." Logic dictated that we expand our armamentarium of assays to include as many enzymes as possible that might be essential to normal red-cell metabolism and longevity. The importance of hexosemonophosphate shunt activity to maintenance of a pool of reducing energy in circulating erythrocytes had already been amply demonstrated by the deleterious effects of glucose-6-phosphate dehydrogenase deficiency. A number of workers in our field recognized that similar defects in enzymes mediating glutathione synthesis and cycling might reasonably be expected to induce chronic or acute episodic hemolysis as a conse-

quence of ambient or drug-induced oxidative stresses.

The seminal work of Gordon C. Mills^{2,3} at the University of Texas focused our attention on glutathione peroxidase specifically, and that was reinforced by an important study by Gerald Cohen and Paul Hochstein⁴ indicating that this peroxidase (rather than catalase) was the primary pathway for hydrogen peroxide detoxification in erythrocytes under physiological conditions. The quantitative assay we developed was essentially a modification of one devised by Mills that relied on periodic assays of residual glutathione after prolonged incubations.³ Valentine suggested that we might be able to develop a direct spectrophotometric assay by linking the peroxidase reaction to glutathione reductase rather than measuring residual substrate. This approach had been tried previously but was associated with unacceptably high blank activities because of the effects of peroxide on hemoglobin. The key to success was pretreatment of the hemolysate with Drabkin's reagent to produce stable cyanmethemoglobin, eliminating methemoglobin-reductase-mediated (or non-enzymatic) oxidation of NADPH. This system had the added advantage of recycling oxidized glutathione immediately back to the reduced form, thereby maintaining a constant concentration of substrate and allowing accurate kinetic studies to be performed. The procedure was simple and reliable and provided a quantitative assay that could be followed in real time by monitoring the conversion of NADPH to NADP with a recording spectrophotometer. The method was quickly adopted by others and continues to be used with various modifications, thus likely accounting for its citation frequency.

To date, no defects in glutathione peroxidase have been unequivocally incriminated in the pathogenesis of hemolytic syndromes, although several instances of partial deficiency have been reported in patients with anemias of unknown etiology. This association may be coincidental, since there is a broad range of ethnic variation in the erythrocyte enzyme.⁵ Ironically, a recent study by G.F. Gaetani *et al.*⁶ provides strong evidence to dispel the traditional view of the relative roles of catalase and glutathione peroxidase in peroxide detoxification established by the Cohen and Hochstein report.⁴

[Editor's note: In *Current Contents* (3):15, 16 January 1989, part of a letter from D.E. Paglia was published. This letter provided additional information about the role of this *Classic* paper in Professor Paglia's very interesting career.]

1. Tanaka K R, Valentine W N & Miwa S. Pyruvate kinase (PK) deficiency hereditary nonspherocytic hemolytic anemia. *Blood* 19:267-95, 1962. (Cited 290 times.) [See also: Tanaka K R. Citation Classic. (Barrett J T, ed.) *Contemporary classics in clinical medicine*. Philadelphia: ISI Press, 1986. p. 162.]
2. Mills G C. Hemoglobin catabolism. I. Glutathione peroxidase, an erythrocyte enzyme which protects hemoglobin from oxidative breakdown. *J. Biol. Chem.* 229:189-97, 1957. (Cited 265 times.)
3. ———. The purification and properties of glutathione peroxidase of erythrocytes. *J. Biol. Chem.* 234:502-6, 1959. (Cited 170 times.)
4. Cohen G & Hochstein P. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry—USA* 2:1420-8, 1963. (Cited 390 times.)
5. Beutler E & Matsumoto F. Ethnic variation in red cell glutathione peroxidase activity. *Blood* 46:103-10, 1975. (Cited 40 times.)
6. Gaetani G F, Galliano S, Canepa L, Ferraris A M & Kirkman H N. Catalase and glutathione peroxidase are equally active in the detoxification of hydrogen peroxide in human erythrocytes. *Blood*. (In press.)

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