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Cohen G & Hochstein P. Glutathione peroxidase: the primary agent for the elimination of hydrogen peroxide in erythrocytes. *Biochemistry—USA* 2:1420-8, 1963.
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When hydrogen peroxide is added slowly by vapor state diffusion to human erythrocytes, cellular catalase fails to protect against progressive oxidative damage. Protection requires glutathione peroxidase, which is sustained by the generation of NADPH via glucose metabolism. [The *SCI*® indicates that this paper has been cited in more than 430 publications.]

Bringing an Enzyme out of the Closet

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The prevailing wisdom in 1960 was that the enzyme catalase protected cells from oxidative damage by hydrogen peroxide (H_2O_2). Our experiments proved that this vital role is best assigned to glutathione peroxidase (GSH-Px).

Gordon C. Mills[1] discovered GSH-Px in 1957, but its implicit role was not accepted. Catalase reigned as the undisputed cell protector. A simple experiment shows why: When a bolus of 3 percent H_2O_2 is added to a test tube containing heparinized blood, the view is one of effervescence (O_2 bubbles) as a foam of protein rises up and overflows the tube. When blood in a second tube is treated with azide to inhibit cellular catalase and then the H_2O_2 is delivered, the red blood cells turn brown-to-black as hemoglobin is rapidly oxidized and effervescence is virtually absent. The seemingly obvious conclusion is that catalase is a prime protector, and the contribution by GSH-Px, if any, is minimal.

We were working with redox-active compounds that generated H_2O_2 and had evidence that protection was linked to glucose metabolism, rather than catalase activity. We were attracted to the unfolding story of drug-induced hemolysis linked to a genetic deficiency in glucose-6-phosphate dehydrogenase (G-6-PD) and associated with oxidation of glutathione and hemoglobin. Because the hexose shunt in erythrocytes is the sole source of NADPH-reducing equivalents required by glutathione reductase, we reasoned that failure to recycle oxidized glutathione could result in loss of protection by GSH-Px. Oxidative hemolysis might be a consequence of failure to detoxify H_2O_2. But, how could this be in the face of the compelling evidence for the preeminence of catalase?

The break came at a FASEB meeting when we heard Irwin Fridovich describe experiments on the H_2O_2-initiated polymerization of sulfite. He used a vapor-state diffusion method to maintain a steady exposure to low-levels of reagent H_2O_2. We thought this approach might better mimic the slow generation of H_2O_2 by oxidant drugs. Perhaps a slow addition of H_2O_2 would give a different picture. It did.

When H_2O_2 diffused from the center well of sealed flasks to suspensions of our own washed red blood cells, GSH was progressively oxidized, despite the presence of active catalase, and this was followed by oxidation of hemoglobin and overt lysis. Stoichiometric studies verified the quantitative importance of GSH oxidation. When glucose was added, cells were protected from damage by H_2O_2 vapor. We reported separately that G-6-PD-deficient erythrocytes lost GSH and exhibited oxidized hemoglobin even in the presence of glucose.[2] A final set of experiments solidified the physiologic importance of GSH-Px. Subjects with a rare genetic error, acatalasia, have blood that turns brownish-black when a wound is swabbed with H_2O_2. The common duck is also acatalasemic. We showed that duck erythrocytes, as well as azide-treated normal human erythrocytes, were protected from diffusion H_2O_2 by glucose. The inescapable conclusion: Catalase is not essential, and GSH-Px protects cells from slowly generated H_2O_2.

As often happens with studies that go against the grain of accepted thinking, our work did not have an easy time making its way into print. Over the years, however, it has become well established that the seleno-enzyme GSH-Px, which detoxifies both H_2O_2 and lipid peroxides, plays a primary role in preventing oxidative cellular damage.

1. 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 280 times.)
2. Cohen G & Hochstein P. Glucose-6-phosphate dehydrogenase and detoxification of hydrogen peroxide in human erythrocytes. *Science* 134:1756-7, 1961. (Cited 125 times.)

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