The Serum Thiol Test: Evidence Supporting the Contention That Serum Thiol Concentrations Are Predictive of DNA Repair Capacity

Overview of the Serum Thiol Test Methodology

The thiol test is performed on the serum component of whole blood which, when exposed to 80% saturated ammonium sulfate, will precipitate thiols present in serum proteins (Banne et al, 2002; Pero, 2003). The sample is then ultracentrifuged (12,000 RPM for 15 minutes) to access the protein-rich fraction, mixed with DTNB (5,5'-dithiobis [2-nitrobenzoic acid]), and the absorbance measured in a spectophotometer at 412 nm.

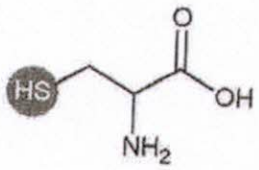
Rational for the Use of Serum Thiol Concentrations as a Measure of DNA Repair Capacity

Serum Thiol, Oxidative Stress, and DNA Repair: The chemical basis of the serum thiol test is the variability in the absorbance of DTNB (Pero, 2003). The stock reagent is colorless when oxidized, but when reduced it becomes yellow and absorbs at 412 nm. The preparation of the specimen results in the precipitation and concentration of blood proteins.

Oxidative stress is a physiologic condition wherein the production of oxidants (free radicals, reactive oxygen species and intermediates, active oxygen species, etc.) exceeds the ability of the organism to neutralize them (Azzi et al, 2004; Turrens, 2003). The abundance or lack of oxidative stress is a continual dynamic process and can be assessed indirectly by a variety of measures, including evidence of oxidative DNA damage (8-hydroxy-2'-deoxyguanine), 8-epi-prostaglandin $F_{2\alpha}$, non-cyclooxygenase-derived prostanoids, and ascorbate free radical. Oxidative stress has been proposed as a factor in the development and propagation of various conditions including asthma, atherosclerosis, inflammatory conditions, certain cancers, and aging (Wood et al, 2003; Young and Woodside, 2001).

The serum thiol test's innovator contends that serum thiol concentration is an *in vivo* measure of reduction-oxidation balance, and, as such is an indicator of the extent of the interconversion of thiol groups (—SH) on the amino acid cysteine (Figure 1) to disulfides (—S-S; Pero, 2004). Further, the oxidative stress-sensitive thiol to disulfide conversion is most

important, from the perspective of human capacity to repair DNA, in its proposed inhibitory effect on the enzyme, poly(ATP-ribose) polymerase (PARP; Pero et al, 1995).



Cysteine

Figure 1. The amino acid cysteine. The thiol group (—SH) is highlighted in white against a red background.

Poly(ADP-Ribose) Polymerase (PARP): PARP has been the subject of more than 30 years of research (Bouchard et al, 2003; Smith, 2001). It was noted early on that PARP activity increased dramatically in cells with damaged DNA. But that observation did not provide a complete picture of PARP, which was subsequently found to be a family of similar proteins; PARP-1, PARP-2, PARP-3, etc. PARP-1, for example, is known to be involved in DNA replication and transcription, apoptosis, and DNA repair (Bouchard et al, 2003). It is a complex protein (1,013 amino acids; Ronen and Glickman, 2001) which can be subdivided into distinct domains (Bouchard et al, 2003). The DNA binding domain is the most important section of PARP-1 with respect to its proposed ability to correlate serum thiol concentration and DNA repair capacity. Contained on the DNA binding domain of PARP-1 are two "zinc fingers," which are essential to recognize damaged DNA. Once bound to a damaged DNA site, PARP-1 recruits other DNA repair enzymes and adds ADP-ribose molecules to DNA proteins (Christmann et al, 2003; Ronen and Glickman, 2001).

The fundamental structure of DNA, the double helix, is physically organized into chromosomes in two turn sections of DNA (approximately 165 base pairs) wound around a cluster of eight proteins (histones; Felsenfeld and Groudine, 2003). This comprises a functional unit of DNA called a nucleosome. Nucleosomes are further packed into chromatin fibers which are folded and condensed into individual chromosomes. This winding and folding of DNA allows the packaging of a large quantity of genetic material into a sufficiently compact size to fit within the cell nucleus. Perhaps of greater importance, when DNA is

wound around histones, areas are not accessible for gene expression, DNA replication, or DNA repair. This is particularly true of the accessibility of DNA to large molecules and complexes that require interactions with multiple base pairs, such as DNA repair (Ura and Hayes, 2002). The PARP-1-mediated ribosylation of DNA allows access to DNA repair enzymes (Christmann et al, 2003).

The Physiologic/Pathophysiologic Basis of the Serum Thiol Test: Thiol groups on the cysteine amino acids in the zinc fingers of PARP-1, in the presence of oxidants (increased oxidative stress), will be chemically altered and unable to normally participate in the DNA repair process (Pero, 1999). Thus, thiol concentration in serum will reflect the chemical integrity of PARP and provide a surrogate measure of DNA repair capacity.

Data Supporting the Contention that Serum Thiol Concentrations Are a Surrogate Measure of DNA Repair Activity

Serum Thiol Concentration and PARP Activity: If serum thiol concentrations are an effective predictor of DNA repair capacity as a surrogate measure of the ability of PARP to participate in DNA repair activities, then serum thiol concentrations should correlate with PARP activity. To assess this contention, plasma was obtained from humans (probably as a component of an open label Phase I/II study of the potential use of metoclopramide as a radiosensitizer in patients with inoperable squamous cell carcinoma of the lung) and each sample (total samples = 225; population size not reported) was divided for two separate analyses (Pero et al, 1995). One of the analyses measured serum thiol concentration as described above, while the other determined the hydrogen peroxide-induced PARP activity in monocytes. The data were found to have a significant linear correlation ($p < 0.001$; $r = 0.506$; Figure 2) and suggest that serum thiol concentration increases with increased PARP activity, presumably in response to DNA damage.

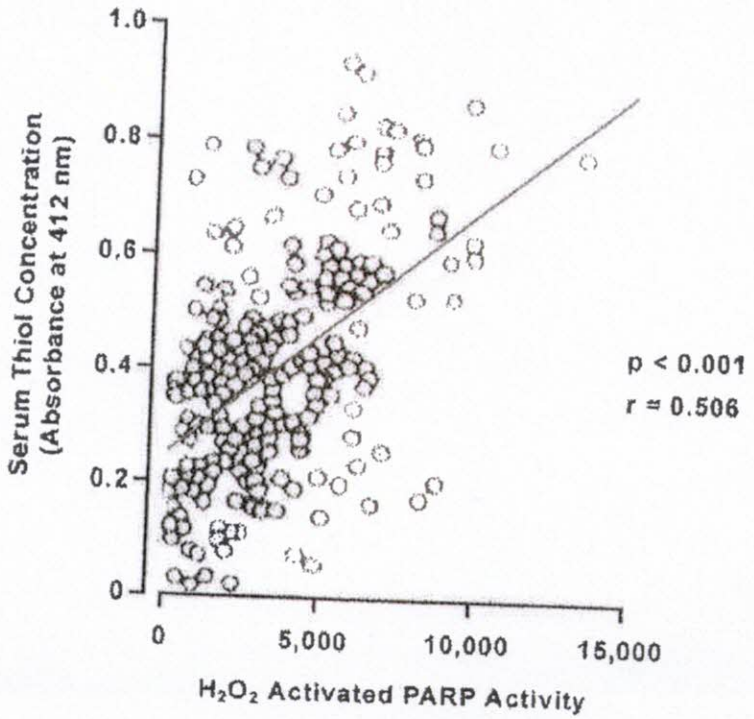


Figure 2. The correlation between hydrogen peroxide-induced (H_2O_2 Activated) PARP activity in monocytes and serum thiol concentrations in humans (Pero et al, 1995). Two hundred and twenty-five samples were plotted.

Serum Thiol Concentrations and Disease: In patients with acute and chronic diseases, an expected increase in oxidative stress, DNA damage, and PARP activation, should result in disparate serum thiol concentrations when compared with healthy individuals. To test that contention, serum thiol concentrations were determined in the blood obtained from 90 apparently healthy individuals between 37 and 70 years of age, and in 306 patients between 34 and 100 years of age with various active diseases (Banne et al, 2003). With the exception of diabetes (n = 26; assumed to be diabetes mellitus) specific diseases were not reported. However, the investigators categorized the balance of the population with active diseases into the following groups: blood disorders (n = 35), cancer (n = 12), cardiovascular disease (n = 69), infectious/inflammatory disease (n = 29), kidney disease (n = 26), metabolic disease (n = 35), neurologic disease (n = 61), and thyroid disease (n = 13). The investigators noted a significant ($p < 0.001$) difference in serum thiol concentrations in healthy controls as compared to patients with active diseases (Figure 3).

The differences were not found to vary because of sex; however, the mean age of patients with active diseases was significantly greater then that of the controls. Thus, the study population was subdivided and reanalyzed after accounting for age and serum thiols and were again found to significantly differ ($p < 0.003$) in healthy controls ($n = 38$) and in patients with active diseases ($n = 125$; Figure 4).

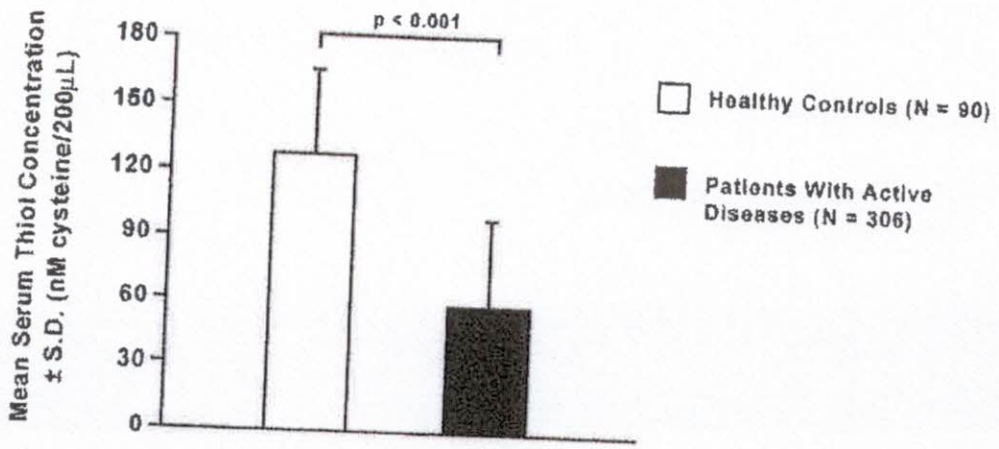


Figure 3. Serum thiol concentrations in healthy individuals and in patients with active diseases (Banne et al, 2003).

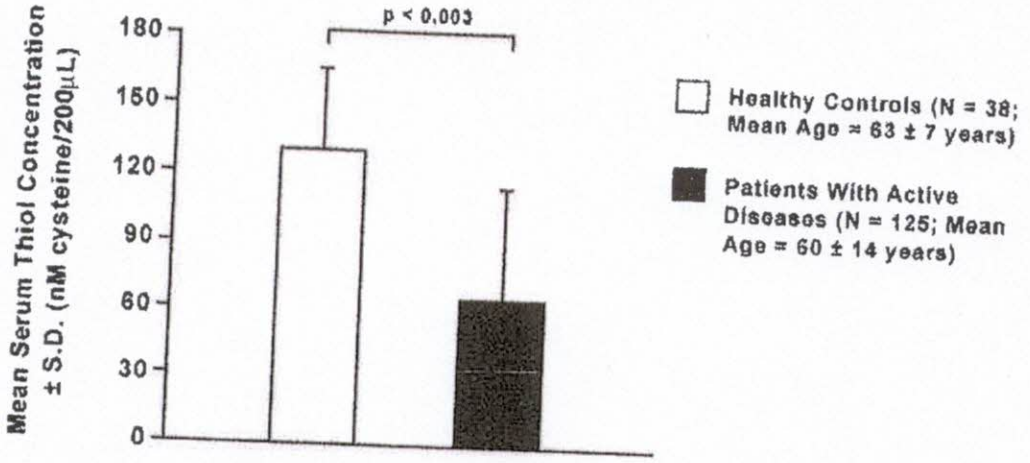


Figure 4. Serum thiol concentrations in the healthy individuals and in patients with active diseases. The groups were age matched to account for the statistical difference in the mean age of the groups when the total population was analyzed (Banne et al, 2003).

In patients with severe diseases, the degree of oxidative stress, DNA damage, and PARP activation, should result in disparate serum thiol concentrations when compared with less sick individuals with the same disease. To test that contention, serum thiol concentrations were determined in 133 HIV-infected intravenous drug users (Marmor et al, 1997). Patients were recruited between 1990 and 1991 and deaths recorded through December of 1993. An age adjusted analysis revealed that patients with low serum thiols had a significantly increased hazard of death than those patients with high serum thiols. Survival at 2 years was 93.9% in patients with high serum thiols (absorbance value at 412 nm > 0.46) and 76.5% in patients with low serum thiols (absorbance value at 412 nm < 0.46).

Serum Thiol Concentrations and Longevity in Mammals: Data suggest that lifespan is influenced by the inherent capacity of a species to repair DNA and by the relative activity of PARP (Cortopassi and Wang, 1996; Grube and Bürkle, 1992). Serum thiol measurements, therefore, if capable of assessing DNA repair capacity, should vary relative to species longevity. To test that contention, a total of 47 serum thiol measurements were determined in the blood of 17 different mammalian species (Pero et al, 2000; Table 1). Blood was obtained from adults of both

sexes and literature reports on longevity, rather than the actual ages of the specimens, were used in the analyses. The investigators determined that mammalian life span and serum thiol concentrations were significantly correlated ($p < 0.001$; $r = 0.841$). This, they argue, supports the contention that aging is negatively impacted by oxidative stress-induced DNA damage and is reflected in serum thiol measurements as a surrogate measure of DNA damage.

Table 1. Data Used to Correlate Mammalian Longevity and Serum Thiol Concentrations (Pero et al, 2000).

Species	Samples Used to Determine Serum Thiol Concentration (n)	Longevity* (years)
Mouse	1	2.0
Rat	1	3.3
Wolf	1	10.0
Dog	2	12.0
Goat	1	12.0
Sheep	2	12.0
Rabbit	1	13.0
Bear	1	14.0
Cat	1	15.0
Lynx	1	15.0
Musk Ox	1	20.0
Fallow Dear	1	20.0
Cow	2	30.0
Gorilla	2	>39.3†
Horse	2	46.0
Chimpanzee	2	>46.6†
Human	25	95.0

* The investigators obtained these data from the literature.
† Recorded as minimal life spans in the literature. It is unclear how the investigators accounted for this in their analyses.

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