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Review Article

REDOX ENVIRONMENT OF THE CELL AS VIEWED THROUGH THE REDOX STATE OF THE GLUTATHIONE DISULFIDE/GLUTATHIONE COUPLE

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Abstract—Redox state is a term used widely in the research field of free radicals and oxidative stress. Unfortunately, it is used as a general term referring to relative changes that are not well defined or quantitated. In this review we provide a definition for the redox environment of biological fluids, cell organelles, cells, or tissue. We illustrate how the reduction potential of various redox couples can be estimated with the Nernst equation and show how pH and the concentrations of the species comprising different redox couples influence the reduction potential. We discuss how the redox state of the glutathione disulfide-glutathione couple (GSSG/2GSH) can serve as an important indicator of redox environment. There are many redox couples in a cell that work together to maintain the redox environment; the GSSG/2GSH couple is the most abundant redox couple in a cell. Changes of the half-cell reduction potential (E_{hc}) of the GSSG/2GSH couple appear to correlate with the biological status of the cell: proliferation $E_{hc} \approx -240$ mV; differentiation $E_{hc} \approx -200$ mV; or apoptosis $E_{hc} \approx -170$ mV. These estimates can be used to more fully understand the redox biochemistry that results from oxidative stress. These are the first steps toward a new quantitative biology, which hopefully will provide a rationale and understanding of the cellular mechanisms associated with cell growth and development, signaling, and reductive or oxidative stress. © 2001 Elsevier Science Inc.

Keywords—Glutathione, NADPH, Nernst equation, Reduction potentials, Free radicals

INTRODUCTION

I often say that when you can measure what you are speaking about and express it in numbers you know something about it; but when you cannot measure it, when you cannot express it in numbers, your knowledge is of a meagre and unsatisfactory kind.

—Lord Kelvin in Popular Lectures and Addresses, lecture on Electrical Units of Measurement, 1883

It is now realized that the direction of many cellular processes depends on "redox state." But at present the term *redox state* is not well defined. The research in this area is mostly observational in that cells or tissues are subjected to an oxidative or reductive stress and then the effects are observed. The research community has not yet related the applied stresses to quantitative changes in redox environment, only to qualitative changes. Thus, we do not know on a quantitative basis the "redox environment" needed to initiate a particular set of cellular signals. In this work we (i) provide a definition for redox environment; (ii) provide a definition of redox state; (iii) show how the Nernst equation can be a tool to provide quantitative estimates of redox state; (iv) review important biological redox couples that play a role in determining the cellular redox environment; (v) illustrate how glutathione uniquely contributes to the cellular redox environment; (vi) examine how protein sulfhydryl groups participate in these processes; and (vii) present a framework for how the redox state of the GSSG/2GSH couple and the biological status of a cell are linked. This framework leads to the proposal that the biological status of a cell is intertwined with its redox environment.

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REDOX STATE AND REDOX ENVIRONMENT, DEFINITIONS

Life depends on overcoming entropy. Energy is required to maintain the ordered state of a living organism.

For humans, this is achieved by capturing the energy released in oxidation processes to: (i) build cellular and organismic structures, (ii) maintain these structures, and (iii) provide the energy for the processes they support. The energy comes from the movement of electrons from oxidizable organic molecules to oxygen. This results in an overall reducing environment in cells and tissues. Redox couples in cells are, of course, responsive to electron flow, that is, changes in the reducing/oxidizing environment. Some of these redox couples are linked to each other to form a set of related couples. Sets of couples can be independent from other sets if activation energies for reactions are high and there are no enzyme systems to link them kineticly. The redox environment of a cell is a reflection of the state of these couples. Bücher was the originator of studies that addressed cellular redox biochemistry [1]. His laboratory developed approaches to determine the states of various redox couples in cells and was the first to estimate the actual cellular reduction potentials (*Ist-Potential*) for the NAD^+/NADH and $NADP^+$/NADPH couples.

Redox state is a term that has historically been used to describe the ratio of the interconvertible oxidized and reduced form of a specific redox couple. For example, Sir Hans Krebs focussed on the NAD^+/NADH couple and defined the redox state of this couple in a cell to be [free NAD^+]/[free NADH] [2–4]. In recent years, the term redox state has been used not only to describe the state of a particular redox pair, but also to more generally describe the redox environment of a cell. This more general use of the term redox state is not very well defined and differs considerably from historical uses. We suggest that the term redox environment be used when a general description of a linked set of redox couples is intended. A definition would be:

The *redox environment* of a linked set of redox couples as found in a biological fluid, organelle, cell, or tissue is the summation of the products of the reduction potential and reducing capacity of the linked redox couples present.

Reduction potential can be thought of as a voltage and reducing capacity would be total charge stored, that is, number of electrons available. Reducing capacity would be estimated by determining the concentration of the reduced species in a redox couple; the reduction potential can be estimated with the Nernst equation. In mathematical terms this could be represented by:

$$\text{redox environment} = \sum_{i=1}^{n\ (\text{couple})} E_i \times [\text{reduced species}]_i \quad (1)$$

where, E_i is the half-cell reduction potential for a given redox pair and $[\text{reduced species}]_i$ is the concentration of the reduced species in that redox pair. It may be impractical to measure all linked redox couples present in biological settings to determine the redox environment. Instead, a representative redox couple could be used as indicator for changes in the redox environment. For example, in the cell the GSSG/2GSH couple provides a very large pool of reducing equivalents. It is considered to be the cellular redox buffer. Therefore, the redox state of this couple could be used as an indicator for the redox environment of the cell.

As mentioned above, the term redox state has been historically used to describe the ratio of the interconvertible oxidized and reduced form of a specific redox couple, for example, [free NAD^+]/[free NADH]. However, this definition needs to be broadened so that it is applicable to all redox couples, such as the GSSG/2GSH couple. As discussed below, to have full knowledge of the redox state of the GSSG/2GSH couple, absolute concentrations are needed. This is in contrast to the NAD^+/NADH or $NADP^+$/NADPH couples where only the ratio of the interconvertible oxidized and reduced form of these redox couples is enough. Thus, the definition of the term redox state needs to contain reduction potential. Another important factor for the redox state is the reducing capacity of the redox couple, that is, how large is the pool for the redox buffering system. Combining both reduction potential and reducing capacity, a definition for redox state would be:

The *redox state* of a redox couple is defined by the half-cell reduction potential and the reducing capacity of that couple.

A convenient notation for the status of a redox pair, such as GSSG/2GSH, would be $\{E_{hc}(GSH); [GSH]\}$, e.g., $\{-180$ mV (GSH); 3.5 mM$\}$.

HOW TO CALCULATE REDUCTION POTENTIALS OF REDOX REACTIONS

The Nernst equation

In 1889 Walter H. Nernst investigated the theory of galvanic cells and developed what is now known as the *Nernst Equation*. The Nernst equation allows one to determine the voltage of an electrochemical cell (ΔE) taking the Gibbs energy change (ΔG) and the mass action expression (Q) into account (Reactions 2–5). The Nernst equation has broad applications in biology because much of biology involves electron transfer reactions. These reactions are responsible for producing en-

ergy and for building and maintaining structures needed by an organism.

The voltage of an electrochemical cell is directly related to the Gibbs energy change.

$$\Delta G^\circ = -n\mathbf{F}\Delta E^\circ, \tag{2}$$

where n is the number of electrons exchanged in the chemical process, **F** is the Faraday constant, and ΔE° is the electromotive force under standard conditions, that is, the difference in the standard reduction potentials of the two half-cells involved in the process. The superscript ° implies the thermodynamic standard state.[1] Life, however, occurs under nonstandard conditions. Under nonstandard conditions the relationship can be derived from a process such as:

$$aRed_1 + bOx_2 \leftrightharpoons cOx_1 + dRed_2 \tag{3}$$

$$\Delta G = \Delta G^\circ + RT \ln Q \tag{4}$$

$$Q = \frac{[Ox_1]^c\,[Red_2]^d}{[Red_1]^a\,[Ox_2]^b} \tag{5}$$

where Q is the mass action expression; RT ln Q is the "correction" factor for being at nonstandard conditions.

Using $\Delta G = -n\mathbf{F}\Delta E$, $\Delta G^\circ = -n\mathbf{F}\Delta E^\circ$, and Eqns. 4 and 5, the voltage of an electrochemical cell can be expressed as:

$$\Delta E = \Delta E^\circ - \frac{RT}{n\mathbf{F}} \ln Q \qquad \textbf{Nernst Equation} \tag{6a}$$

where R is the gas constant ($R = 8.314\ J\ K^{-1}\ mol^{-1}$), T the temperature (in Kelvin), and **F** the Faraday constant ($\mathbf{F} = 9.6485 \times 10^4\ C\ mol^{-1}$). This will yield results in volts. The Nernst equation at T = 25°C (298.15 K), using 2.303 as the conversion factor for ln into $\log_{10}$, can be written as:

$$\Delta E = \Delta E^\circ - \frac{59.1\ mV}{n} \log Q \tag{6b}$$

The Nernst equation at T = 37°C (310 K) is:

$$\Delta E = \Delta E^\circ - \frac{61.5\ mV}{n} \log Q \tag{6c}$$

Thus, with the Nernst equation the reduction potential between two redox couples (electrodes) in an electrochemical cell can be estimated.

Redox reactions and equilibrium

From a thermodynamic point of view, a cell is clearly not in equilibrium with the environment. Equilibrium with the environment will be achieved when a cell dies, $\Delta E = 0$. That is, the reduction potential of all redox-pairs in the cell will shift to the potential of the O_2/H_2O redox pair ($E^{\circ\prime} = +0.82$ V at pH 7.0). Inside a cell the chemical reactions attempt to achieve equilibrium. Only selected reactions are allowed freely to proceed toward equilibrium, for example, sugars + $O_2 \rightarrow CO_2 + H_2O$. However to maintain structure and function many possible reactions are not allowed to proceed toward equilibrium; they are maintained in a nonequilibrium steady state. Therefore, over short periods of time, the concentrations of these redox pairs and the ratios [Ox]/[Red] do not change, and the Nernst equation can be applied:

Redox-pair 1	$Red_1 \rightarrow Ox_1 + e^-$
Redox-pair 2	$Ox_2 + e^- \rightarrow Red_2$
Redox-reaction	$Red_1 + Ox_2 \rightleftharpoons Ox_1 + Red_2$

The electromotive force from these redox pairs is $\Delta E = E_2 - E_1$ (E_2 will be the reduction potential for the half-reaction of the species that is reduced and E_1 is the reduction potential for the half-reaction of the species that is oxidized in the overall reaction).

$$\Delta E = \left(E_2^\circ - \frac{59.1\ mV}{n} \log \frac{[Red_2]}{[Ox_2]}\right) - \left(E_1^\circ - \frac{59.1\ mV}{n} \log \frac{[Red_1]}{[Ox_1]}\right) \tag{7}$$

Each redox pair forms an electrochemical half-cell. The electromotive force ΔE is the difference in the reduction potential of the half-cells of a system. When ΔE is zero, there is no net electron flow.

We do not have a definite battery in biology and we really are not at equilibrium. But by using the Nernst equation we can still make estimates of the half-cell reduction potentials for various redox couples in biological settings.

Redox processes and pH

Many redox reactions are pH-dependent. Thus, the concentration of H^+ needs to be considered, as it can

[1]Standard conditions: E°, G°. . . ° imply 1 molal solution (unit activity); 1 atm pressure for gases; T = 298 K or 25°C and pH = 0. If a nonstandard condition is to be used as a reference state, such as the pH being 7, then a prime mark (′) is added to these notations, i.e., E°′, G°′, etc. All E° and E°′ are measured against the normal hydrogen electrode. This electrode by convention is defined to have E° and E°′ = 0 mV. A potential for the half reaction: Ox + ne^- → Red is a reduction potential; an oxidation potential corresponds to Red → Ox + ne^- as a half reaction (see reference [9]). In this work all values of E are reduction potentials unless otherwise noted.

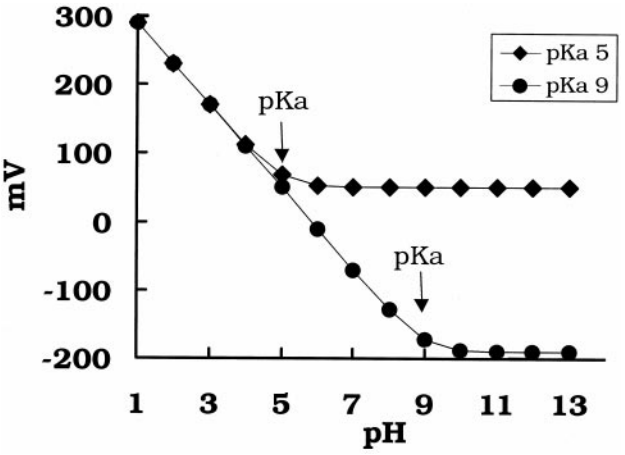


Fig. 1. The reduction potential of redox couples can be pH dependent. Shown are two examples of how the reduction of two different redox pairs change versus pH. The two examples have quite different pK_as, but the half-cell potentials at pH = 0 are the same. Data were derived from Eqn. 10 using a half-cell potential of +350 mV at pH 0.

change the half-cell potential dramatically. For example, for the half-reaction:

$$A + e^- + H^+ \rightleftharpoons AH^\bullet \tag{8}$$

if the species $AH^\bullet$ is an acid, then:

$$AH^\bullet \overset{pK_a}{\rightleftharpoons} A^{\bullet -} + H^+ \tag{9}$$

If [total radical] = $[A^{\bullet -}]$ + $[AH^\bullet]$, then the pH dependence can be demonstrated by a simplified Nernst equation:

$$E_m = E_0 - 59.1\ \text{mV} \log\left(\frac{1}{K_a + [H^+]}\right) \text{ at } 25°C \tag{10}$$

where E_m is the midpoint potential for the redox-couple $A/A^{\bullet -}$, that is, when [oxidant] = [reductant], E_0 is the potential with $[H^+]$ = 1 M, and n = 1. Figure 1 shows this pH dependency for two different couples, one where $AH^\bullet$ has a pK_a of 5 and the other where pK_a is 9. Note, that if the pK_a is above physiological pH (pH 7.4), then changes in pH can bring about changes in the reduction potential for a couple. But if the couple has no acid hydrogens involved or if the pK_a is significantly below physiologic pH, then reasonable changes in cellular pH will have no influence on E_{hc} for this redox couple. The example above, Eqns. 8–10, has only one proton and, of course, one pK_a involved in the prototropic equilibrium. There are other scenarios, such as two protons with two different pK_as, for example, ascorbic acid and catechols or two protons with one pK_a such as the glutathione disulfide/glutathione couple. (For an excellent concise presentation of the influence of pH on reduction potential see the discussion by Wardman [5].) For simplicity, the most practical approach for using the Nernst equation to estimate E_{hc} at different pH values is to adjust the E°′ to the value at the pH of interest. For example, for a two-electron reaction:

$$A + 2H^+ + 2e^- \rightleftharpoons AH_2 \tag{11}$$

the Nernst equation can be written as:

$$E_{pH} = E° - \frac{59.1\ \text{mV}}{n} \log \frac{[AH_2]}{[A]\,[H^+]^2} \qquad \text{and } n = 2 \tag{12}$$

where, E_{pH} is the half-cell reduction potential at a particular pH. There are two ways to calculate E_{pH}. For example, if we assume standard-state concentrations for A and AH_2, that is 1 M and standard temperature 25°C (298 K), then the two approaches are:

1) E° is the potential at standard conditions (pH = 0), so the actual $[H^+]$ is inserted into Eqn. 12 and then solved. For example the $E°_{pH=0}$ for the GSSG/2GSH couple is approximately +180 mV. At pH 7, that is, $[H^+] = 10^{-7}$, $E_{pH=7}$ (or E°′) is −240 mV, $E_{pH=7.4}$ = −264 mV, and $E_{pH=8}$ = −299 mV.
2) Because for biological compounds the values of E°′ (pH = 7) are commonly tabulated, the calculation of E_{pH} is easily achieved using E°′:

$$E_{pH} = E°′ + ((\text{pH} - 7.0) \times (\Delta E/\Delta \text{pH}))\ \text{mV} \tag{13}$$

where, ΔE/ΔpH is the change in E if the pH is increased by 1 unit, Table 1.

Using the GSSG/2GSH couple again as an example, at 25°C:

$$E_{7.4} = -240 + [(7.4 - 7.0) \times -59.1]\ \text{mV} = -264\ \text{mV} \tag{14}$$

The value of ΔE/ΔpH is dependent on the number of electrons and the number of protons involved in the process (see Table 1). Thus, pH must be considered when estimating the redox state of a redox couple. Shifts in pH can have different consequences in cells: (i) if the cellular pH shifts, then to maintain a given value of the reduction potential the ratio of the concentration of pH-dependent redox couples would have to change; or (ii) a shift in pH could be counterbalanced by an appropriate shift in the reduction potential of certain redox couples, so that important ratios such as $[NAD^+]$/[NADH] can remain unchanged. For example, if the pH increases, then a decrease in the ratio of [lactate]/[pyruvate] can

Table 1. Changes in E if the pH is Increased by 1 unit (ΔE/ΔpH)

Number of H^+	Reaction	ΔE/ΔpH[a] (mV) 25°C	ΔE/ΔpH[a] (mV) 37°C	Example	pK_a	Ref.
0	$Ox + 2e^- \leftrightarrow Red^{2-}$	0	0		none	
1	$Ox + e^- + H^+ \leftrightarrow Red$	−59.1[b]	−61.5[b]	$Asc^{\bullet-}$, $H^+/AscH^-$	−0.7/4.2/11.6[c]	[150]
1	$Ox + 2e^- + H^+ \leftrightarrow Red^-$	−29.6	−30.8	$NADP^+$, H^+/NADPH	>10.5[d]	[20,151]
2	$Ox + 2e^- + 2H^+ \leftrightarrow 2Red$	−59.1	−61.5	GSSG, $2H^+$/2GSH	8.92[e]	[65]
2	$Ox + 2e^- + 2H^+ \leftrightarrow Red$	−59.1	−61.5	TrxSS, $2H^+/Trx(SH)_2$	if pK_{SH} > physiol pH	

[a] Keep in mind that the pH of interest must be less than the applicable pK_a to use these values, otherwise ΔE/ΔpH = 0, see Fig. 1.
[b] These values for ΔE/ΔpH will hold in the physiological pH range for ascorbate, the example, i.e., pH > 4.2.
[c] These pK_as are for the species $AscH^{\bullet}$, $AscH_2$, and $AscH^-$, respectively.
[d] This is deduced from the data of Rodkey and Donovan in that there is no evidence of a pK_a in the curve of E vs. pH over the range 6.2 to 10.5, a straight line with slope = −30.3 mV per pH unit (30°C).
[e] This pK_a is for the SH-group of GSH; it is dependent on ionic strength. Both the amino and carboxyl groups are involved. At physiological pH the carboxyl groups are ionized while the amino group is mostly protonated; the pK_a for the thiol is 8.92 at ionic strength of 160 mM [152,153]. The more commonly quoted pK_a of the thiol group is 9.2. The value 9.2 is the pK_a of 8.92 extrapolated to zero ionic strength.
This table was adapted with minor changes from ref. [6].

counterbalance the pH shift, preserving the important ratio of $[NAD^+]/[NADH]$ [6].

REDOX REACTIONS IN BIOLOGY, SOME EXAMPLES

1e⁻-process

The redox reactions of superoxide in typical biological settings are $1e^-$-processes. The Nernst equation for the $O_2/O_2^{\bullet-}$ redox pair would be:

$$E_{hc} = E^{\circ\prime} - 59.1 \log [O_2^{\bullet-}]/[O_2] \text{ mV} \quad \text{at 25°C, pH 7.0} \quad (15a)$$

where, $E^{\circ\prime}_{O_2/O_2^{\bullet-}} = -160$ mV[2] [7,8,9] and E_{hc} is the half-cell reduction potential. For example, if the steady-state level of superoxide in a cell is 10^{-10} M and dioxygen is 10 μM (10^{-5} M), then:

$$E_{hc} = -160 \text{ mV} - 59.1 \log (10^{-10}/10^{-5}) \quad \text{at 25°C, pH 7.0} \quad (15b)$$

$$E_{hc} = +136 \text{ mV}.$$

This positive potential implies that this half-reaction could easily push toward forming superoxide by another redox pair. The ubiquinone (CoQ)/ubisemiquinone radical ($CoQ^{\bullet-}$) in the mitochondrial electron transport chain is a candidate redox pair. This couple may be a source of superoxide production in cells and tissues [10–12]. The reaction would be:

$$CoQ^{\bullet-} + O_2 \rightleftharpoons CoQ + O_2^{\bullet-} \quad (16)$$

$$E^{\circ\prime}(CoQ/CoQ^{\bullet-}) = -40 \text{ mV [5]} \quad (17)$$

The Nernst equation for this system is:

$$\Delta E = \{ -160 \text{ mV} - 59.1 \log ([O_2^{\bullet-}]/[O_2])\} - \{ -40 \text{ mV} - 59.1 \log ([CoQ^{\bullet-}]/[CoQ])\} \quad (18a)$$

$$\Delta E = -120 - 59.1 \log \frac{[O_2^{\bullet-}]\,[CoQ]}{[O_2]\,[CoQ^{\bullet-}]} \text{ mV} \quad (18b)$$

If the ratio of $[O_2^{\bullet-}]/[O_2]$ is $10^{-10}/10^{-5}$, then a ratio of $[CoQ]/[CoQ^{\bullet-}]$ of 1000/1 would yield $\Delta E \approx 0$. The reaction would be at thermodynamic equilibrium; the equilibrium constant for the reaction, as written, is $\approx 10^{-2}$. According to Le Chatelier's principle, decreasing the steady-state level of $O_2^{\bullet-}$ in the mitochondria by increasing manganese superoxide dismutase levels will increase the flux of $O_2^{\bullet-}$ produced in the mitochondria. That this could indeed happen was shown in intact mitochondria using dihydroorotic acid as a source of $O_2^{\bullet-}$ [13]. Antimycin A and thenoyltrifluoroacetate reduced the flux of $O_2^{\bullet-}$ below the limit of detection; addition of SOD to this incubation resulted in an increased flux of $O_2^{\bullet-}$. An increase in the flux of $O_2^{\bullet-}$ would result in an increase in the flux of H_2O_2. Using dichlorofluorescein fluorescence as an indicator of intracellular H_2O_2, increased levels of H_2O_2 have been observed in cells that overexpress MnSOD when compared to controls [14,15].

[2] Two different thermodynamic reference states are used for the $O_2/O_2^{\bullet-}$ couple. In this equation we have chosen to use the aqueous concentration of oxygen, the appropriate reference state is a solution that is 1 molal (≈1 M) in O_2 and $E^{\circ\prime} = -160$ mV. The second reference state often used is 1 atmosphere of O_2; $E^{\circ\prime}$ is then −330 mV. A pressure of 1 atmosphere of O_2 will result in $[O_2] \cong 1.25$ mM in room temperature aqueous solutions. If this reference state is used, then in the Nernst equation P_{O2} replaces $[O_2]$ in all equations. The same value for E will result. The pK_a of $HO_2^{\bullet}$ is 4.7; thus, there is no significant pH dependence of $E^{\circ\prime}$ in the region of pH = 7.4.

2e⁻-process

Most redox reactions in biology are two-electron processes. This ensures that the majority of products from these reactions are closed-shell molecules, thereby avoiding potentially dangerous free radical processes. There is, of course, a thermodynamic hierarchy for these two-electron redox reactions [16–18]. Examples for the use of reduction potentials in understanding redox processes in the free radical/antioxidant network of biological fluids, cells, and tissues follow.

The nicotinamide adenine dinucleotide phosphate system: $NADP^+$/NADPH. NADPH is a major source of electrons for reductive biosynthesis. As such, it is an important electron source for the glutathione system. It provides two electrons[3] as it is oxidized from NADPH to $NADP^+$. Each one-electron process has its own reduction potential. The one-electron steps have been studied for the related NAD^+/NADH couple [5,19]:

$$\text{a. } NAD^+ + e^- \rightarrow NAD^\bullet \quad E^{\circ\prime} = -913 \text{ mV}$$
$$\text{b. } NAD^\bullet + e^- + H^+ \rightarrow NADH \quad E^{\circ\prime} = +282 \text{ mV}$$
$$\text{c. } NAD^+ + 2e^- + H^+ \rightarrow NADH \quad E^{\circ\prime} = -316 \text{ mV}$$

at 25°C, pH 7.0

The value of the Es for the one-electron steps cannot simply be added to estimate the overall reduction potential because the value of E for a process depends on the number of electrons; the Gibbs energy changes for each step must be added:

$$\Delta G_a + \Delta G_b = \Delta G_c$$

or

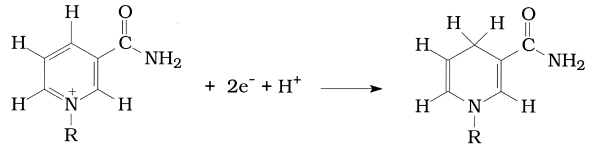
$$-n_a \mathbf{F} E_a^{\circ\prime} + -n_b \mathbf{F}\, E_b^{\circ\prime} = -n_c \mathbf{F}\, E_c^{\circ\prime}$$
$$(-930 + 282)\text{ mV} = -631 \text{ mV}$$

Here, $n_a = n_b = 1$, but $n_c = 2$. Thus, $E_c^{\circ\prime} = -316$ mV. As would be expected, this value is very similar to that of the $NADP^+$/NADPH couple, as determined by Rodkey, $E^{\circ\prime} = -315$ mV [20]. For the $NADP^+$/NADPH couple in an organism the half-cell potential is:

$$E_{hc} = -315 - (59.1/2) \log ([\text{NADPH}]/[\text{NADP}^+])\text{ mV}$$

at 25°C, pH 7.0 (19)

[3]The oxidation of NADPH occurs in the nicotinamide moiety:



The change in the Gibbs energy for the overall reaction will determine if the reaction of NADPH with another redox pair is thermodynamically favorable. One-electron oxidation to form $NADP^\bullet$ is avoided since $NADP^\bullet$ is a very reducing radical, $E^{\circ\prime} = -910$ mV. If dioxygen is present, $NADP^\bullet$ will react very rapidly ($k = 1 \times 10^9$ $M^{-1}s^{-1}$ [21]) to form superoxide. The energetics of each step of the oxidation of NADPH appear to be ideal for catalysis by enzymes. The first step would be facilitated by an enzyme with the second step providing the driving force, both thermodynamically and kinetically, for the oxidation.

As shown above, the real-world reduction potential for the $NADP^+$/NADPH half-cell is dependent on the ratio of the reduced and oxidized forms. A change in this ratio will change the potential of this redox couple, Fig. 2. The half-cell potential for the $NADP^+$/NADPH couple is independent of the absolute concentration of either species, only the ratio is needed to estimate the reduction potential. In cells and tissues the [NADPH]/[$NADP^+$] ratio tends to be approximately 100:1, while the [NADH]/[NAD^+] ratio tends to be between 1:10 and 1:1000 [22]. NADPH is, in general, a cofactor in reductive (biosynthetic) reactions and serves as a source of electrons, while NAD^+-dependent reactions are, in general, oxidative (catabolic) reactions and NAD^+ serves as a sink for electrons. These couples are maintained many orders of magnitude away from equilibrium with each other, allowing them to fulfill these functions. There appear to be cytosolic binding sites that favor the binding of either NADH or $NADP^+$, helping to keep the couples so far from equilibrium [6]. Bücher et al. showed that in rat liver approximately 80 nmol NADH/g wet weight are bound to these cytosolic binding sites [23, 24]. In rat liver [NADPH]/[$NADP^+$] has been estimated to be on the order of 100:1 [25]. Plugging this ratio into the Nernst equation results in a half-cell potential of −374 mV, which is quite reducing. This potential is quite similar to −390 mV determined in liver cytosol [24,26,27]. This reducing nature of the $NADP^+$/NADPH couple in cells makes it an excellent source of electrons. NADPH is considered the primary source of reducing equivalents for the glutathione system, which is especially important to meet an oxidative stress.

The glutathione system: GSSG/2GSH. Glutathione is considered to be the major thiol-disulfide redox buffer of the cell [28]. On average, the GSH concentration in the cytosol is 1–11 mM [29]. This is far higher than most other redox active compounds. Measurements of total

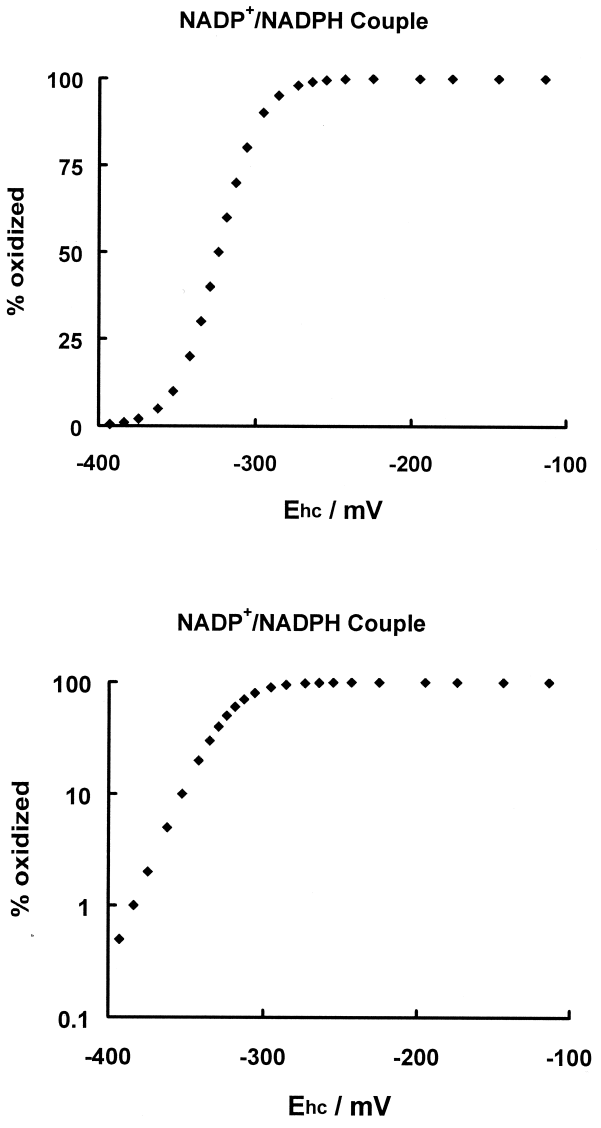


Fig. 2. The reduction potential of the $NADP^+$/NADPH half-cell becomes more positive with increasing $NADP^+$. The midpoint potential of this couple is taken as −315 mV at 25°C and pH 7.0. The linear and log plots provide quite different perspectives on the behavior of this redox pair. Data points are derived from calculations using Eqn. 19.

GSH[4] and/or GSSG levels have been used to estimate the redox environment of a cell. Many researchers estimate the redox state of the system by taking the ratio of [GSH]/[GSSG]. This is convenient as the units divide out, so it is not necessary to determine an absolute concentration. A measurement in μg/mg protein, arbitrary fluorescence units, or the area under an HPLC peak can be entered into the ratio and a useful estimate made.

[4]Total glutathione is traditionally considered to be a measurement of the complete pool of GSH. For the measurement, GSSG is usually reduced to GSH, and then total glutathione is determined. Because 1 GSSG molecule forms 2 GSH upon reduction, total glutathione = $GSH_i + 2\ GSSG_i$, where GSH_i and $GSSG_i$ are the initial levels before reduction. One often sees in the literature total glutathione = $GSH_i + 1/2\ GSSG_i$. This expression is specific to the traditional kinetic assay in which GSH is used to construct a standard curve for the determination of GSSG.

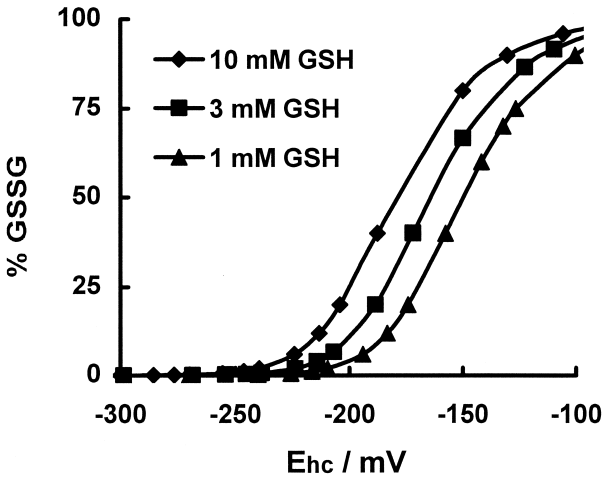


Fig. 3. The reduction potential of the GSSG/2GSH half-cell is dependent on both the ratio of [GSH]/[GSSG] and the concentration of GSH. The ordinate represents the percent GSH that has been oxidized to GSSG. The 10, 3, and 1 mM GSH are initial concentrations of GSH before any is oxidized. For example, if 10% of the GSH for the 3 mM line is oxidized, then [GSH] = 2.7 mM and [GSSG] = 0.15 mM. These plots show that the same ratios of [GSH]/[GSSG] will result in different reduction potentials as the concentration of GSH changes. For example, if 1% of the GSH is oxidized to GSSG, then if we started with 10 mM GSH, $E_{hc} = -250$ mV; but if we start with only 1 mM GSH, then $E_{hc} = -220$ mV. These plots show how the concentration of GSH influences E_{hc} and how [GSH] contributes to the buffering of the cellular redox environment. Data points are derived from calculations using Eqn. 21.

In contrast to the NADPH system however, the absolute concentrations of the components of the GSSG/2GSH redox pair have an impact on the reduction potential. The half-cell reaction is:

$$GSSG + 2H^+ + 2e^- \rightarrow 2GSH. \quad (20)$$

Thus, the Nernst equation for the reduction potential of the GSSG/2GSH half-cell will have the form:

$$E_{hc} = -240 - (59.1/2) \log ([GSH]^2/[GSSG])\ mV \text{ at } 25°C, \text{ pH } 7.0. \quad (21)$$

Note that [GSH] enters as a squared term. This means that the reduction potential is dependent on the GSH/GSSG ratio and the absolute concentration of GSH, Fig. 3. If [GSSG] remains constant but the absolute concentration of GSH changes by a factor of 10, there will be a 59.1 mV change in the reduction potential of the GSSG/2GSH couple. A good example for the importance of calculating E_{hc}, rather than estimating only the GSH/GSSG ratio, is provided by Kirlin et al. [30]. Colon cancer cells were differentiated with sodium butyrate and

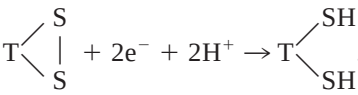
then exposed to benzene isothiocyanate, a compound that induces detoxifying enzymes. Sodium butyrate brought about a 3.6-fold decrease in the GSH to GSSG ratio; addition of benzene isothiocyanate decreased the ratio 3.7-fold. Sodium butyrate changed E_{hc} by 14 mV while benzene isothiocyanate changed E_{hc} by 40 mV. The difference of 26 mV in E_{hc} resulted because when cells were treated with benzene isothiocyanate they had a much smaller GSH pool due to the pretreatment with sodium butyrate.

Thus, in contrast to the estimation of E_{hc} for the $NADP^+$/NADPH couple where absolute concentrations are not needed, that is, only the ratio of concentrations is required for the Nernst equation, the estimation of the redox state of the glutathione couple requires absolute concentrations, that is, molarity of GSH and GSSG. Therefore, the volume of the cells must be determined, which brings with it more work and many new laboratory challenges.

The plots in Fig. 3 show not only how the concentration of GSH influences E_{hc}, but also how [GSH], that is, reducing capacity, contributes to the buffering of the cellular redox environment. For example, if 1% of the GSH is oxidized to GSSG, then if we started with 10 mM GSH, $E_{hc} = -250$ mV; but if we start with only 1 mM GSH, then $E_{hc} = -220$ mV. A cell with 10 mM GSH might be more resistant to oxidative stress than a cell that contains 1 mM GSH. This figure demonstrates that reducing capacity is important in maintaining the redox environment.

The thioredoxin system: TrxSS/Trx(SH)$_2$. Thioredoxin is another important thiol-system in the cell. It reduces cystine moieties in the DNA-binding sites of several transcription factors and is therefore important in gene expression [31,32]. Intracellular concentrations of thioredoxin range from approximately 1 to 10 μM in bovine tissue [33] and up to 15 μM in bacteria [34]. Thus, thioredoxin levels are 100- to 1000-fold less than GSH. The thiols of mammalian thioredoxin serve as electron donors (hydrogen atom) for ribonucleotide reductase as well as for methionine sulfoxide reductase. It also facilitates the refolding of disulfide-containing proteins and regulates the DNA binding activity of some transcription factors [35].

In contrast to the small-molecule antioxidant glutathione that forms inter-molecular disulfides, thioredoxin is a protein that usually forms intra-molecular disulfides. The reduction of the disulfide back to the dithiol form is catalyzed by thioredoxin reductase, the source of electrons being NADPH [36,37].



$$\mathrm{T}\begin{matrix}\mathrm{S}\\|\\\mathrm{S}\end{matrix} + 2e^- + 2H^+ \rightarrow \mathrm{T}\begin{matrix}\mathrm{SH}\\\\\mathrm{SH}\end{matrix} \quad (22)$$

The Nernst equation for the thioredoxin half-cell potential has the same form as that of NADPH:

$$E_{hc} = E^{\circ\prime} - (59.1/2) \log ([T(SH)_2]/[TSS]) \text{ mV} \quad \text{at } 25^\circ\text{C, pH } 7.0 \quad (23)$$

Interestingly, the value of $E^{\circ\prime}$ for the various thioredoxins can vary over a wide range (-270 to -190 mV) [38], depending on the environment of the thiol groups in the protein. This variation in $E^{\circ\prime}$ reflects the ease of oxidation of the thiols in the molecule, which is a function of the pK_a of the thiol(s). The pK_a of cysteine is about 8.5, but the amino acids in the immediate environment of a cysteine in a protein can change this considerably, to as low as 3.5 [39]. Basic amino acids can enhance ionization of the thiol, that is, lower the pK_a. Because, it is the thiolate anion, RS^-, that controls the oxidation of thiols, lower pK_as result in a more positive $E^{\circ\prime}$ and in general a faster rate of oxidation.

The three redox systems $NADP^+$/NADPH, GSSG/2GSH, and TrxSS/Trx(SH)$_2$ are not isolated systems. Both the Trx and GSH-systems use NADPH as a source of reducing equivalents; thus, they are thermodynamically connected to each other. The redox environment of cells and tissues is influenced by the half-cell potentials of these linked redox couples, Fig. 4. This figure clearly shows why NADPH is the thermodynamic driving force for the GSH and thioredoxin systems. Because the concentration of GSH is so much higher than that of the other two systems, it is often considered the principal redox buffer of the cell. This is seen when applying the definition of redox environment. If GSH and NADPH are the dominant source of reducing equivalents for determination of the redox environment, then if we assume that in a typical cell [GSH] $\approx$ 5 mM, E_{hc} (GSSG/2GSH) $= -240$ mV, and [NADPH] $\approx$ 0.1 mM[5] [6] and E_{hc} ($NADP^+$, H^+/NADPH) $= -370$ mV, then from Eqn. 1 above:

$$\text{Redox Environment} = (5 \text{ mM} \times -240 \text{ mV}) + (0.1 \text{ mM} \times -370 \text{ mV})$$

$$\text{Redox Environment} = [(-1200) + (-40)] \text{ mV mM} = -1240 \text{ mV mM}$$

[5]This value is a middle value from estimates in various tissues. For example, skeletal muscle has 27 nmol/g. Liver has a higher level of NADPH, 428 nmol/g wet weight) (see ref. [6]).

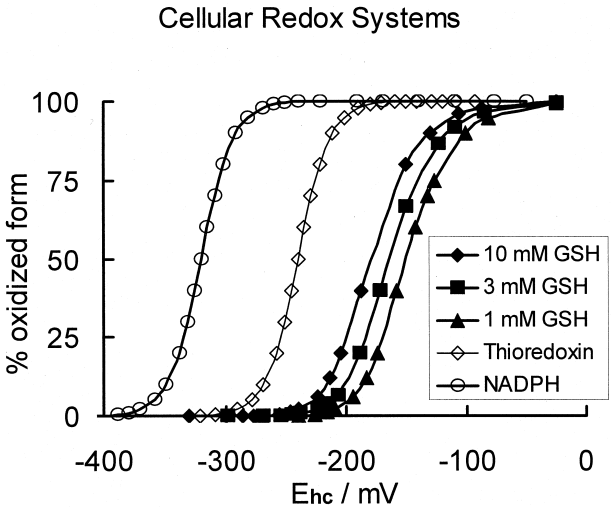


Fig. 4. The $NADP^+$/NADPH couple provides the reducing equivalents needed for the thioredoxin and GSH system. The data are derived from Figs. 2 and 3 and Eqns. 19, 21, and 23. The thioredoxin E°′ is taken as −240 mV. For the $NADP^+$/NADPH couple to be an efficient source of reducing equivalents for the GSH system, these two systems should not be at thermodynamic equilibrium. This clearly is the case as the potential of the $NADP^+$/NADPH couple is considered to be on the order of −375 mV in the cell while that of the GSSG/2GSH couple is on the order of −240 mV (Table 4). Thus, these two redox pairs, which are connected by glutathione disulfide reductase (GR, Fig. 5), appear to be out of equilibrium by a factor of 1000 or more. Whether this is truly the situation needs further investigation.

As seen in this example, the overall redox environment results principally from the contribution of the GSSG/2GSH couple, demonstrating why glutathione is considered to be the major redox buffer in the cell.

GSH AND THE CELLULAR REDOX ENVIRONMENT

Compartmentation of GSH and redox-environment [29]

When dealing with homogeneous fluids such as plasma, the assessment of the redox environment is relatively uncomplicated because the determination of the molar concentrations of GSH and GSSG is straightforward. But when dealing with cells or tissues, compartmentation of GSH and GSSG may pose a problem, as all compartments may be at a nonequilibrium steady-state with respect to each other. A measurement of total content of GSH and GSSG in cells would represent an overall redox environment, not the redox environment of the various compartments such as the endoplasmic reticulum, nucleus, or mitochondria (see Table 2 as example). For many cell types, this overall measurement will represent principally the redox environment of the cytosol, as it is often the largest compartment in a cell, assuming that there are no large gradients of total glutathione between compartments. For example, the nucleus of a hepatocyte has a diameter of about 10 μm while the diameter of the cell is approximately 20 μm [40]. Assuming that the volume of each can be estimated by a sphere, the nucleus is approximately 10–15% of the cell volume; in a nerve cell the nucleus can be $<$ 1% of the cell volume [41]. At the other extreme, the nucleus in T-lymphocytes occupies a large portion of the cell volume, as much as 50% or more [42].

Table 2. Compartmentation of GSH

Location	[GSH]/mM	Ref.
Extracellular	0.010	[29]
	0.8[a]	[154]
	0.002[b]	[155]
Cytosol	1–11	[28,156,157]
Mitochondria	≈5–11	[47,158]
Nucleus	Approx. same or greater than cytosol[c]	[29,49,159]

[a] Alveolar lining fluid.
[b] Human plasma.
[c] The GSH level in the nucleus is, in general, 5–10% of total cellular GSH. 10–20% of this GSH appears to be independent from the cytosol.

GSH and GSSG are found outside cells, but generally in low amounts, Table 2. Extracellular glutathione is thought to function in detoxification processes as well as in providing protection against oxidant injury, but its concentration is usually 100 to 1000 times less than intracellular glutathione.

The majority of GSH in cells is usually found in the cytosol, 1–11 mM [29]. The cytosol also appears to be the principal location of GSH biosynthesis. GSH is synthesized from L-glutamate with the help of two enzymes, γ-glutamylcysteine synthetase (γ-GCS) and glutathione synthetase (GS), Fig. 5. GSH synthesis can be decreased by BSO, which inhibits γ-GCS [43–45].

The cytosol exchanges GSH with intracellular compartments [46–48]. Some organelles however, appear to have their own GSH pools that are independent of the GSH of the cytosol. For example, part of the GSH pool in the nucleus is independent from the cytosol [49]. The independence of this GSH pool from cytosolic GSH is supported by the fact that BSO does not deplete the nuclear GSH pool completely [50,51]. GSH in the nucleus maintains the redox state of critical protein sulfhydryls, which are necessary for DNA repair and expression [52].

In contrast to the mitochondria and the nucleus, the endoplasmic reticulum is more oxidizing than the cytosol. The ratio of GSH/GSSG appears to range from 1:1 to 3:1 [53]. This is quite low compared to the overall ratio in a cell, which is typically greater than 30:1 and usually $\geq$ 100:1. For example, Hwang et al. report that in CRL-1606 cells (murine B-lymphocytes) the E_{hc} for the

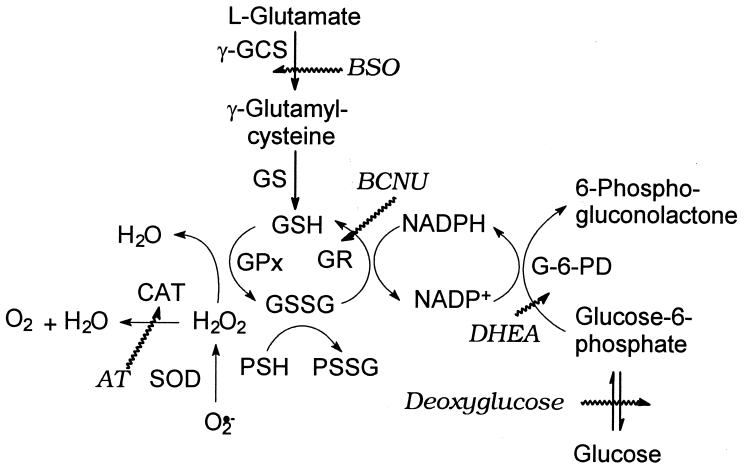


Fig. 5. The GSH system as we know it. This schematic shows the relationships between antioxidant enzymes and glutathione. Compounds shown in italic (with fuzzy arrows) are inhibitors of the GSH system (AT, BCNU, BSO, DHEA, deoxyglucose). Abbreviations: AT = 3-amino-1,2,4-triazole; BCNU = carmustine; BSO = L-buthionine-SR-sulfoximine; CAT = catalase; DHEA = dehydroepiandrosterone; G-6-PD = glucose-6-phosphate dehydrogenase; γ-GCS = γ-glutamylcysteine synthetase; GS = glutathione synthetase; GPx = glutathione peroxidase; GR = glutathione reductase; SOD = superoxide dismutase.

GSSG/2GSH couple in the endoplasmic reticulum is −180 mV, while the cytosol has a value of −232 mV [53]. The more oxidizing environment of the endoplasmic reticulum appears to be needed to produce proteins that have necessary disulfide bonds. Thus, compartmentalization of GSH can result in different redox environments in these compartments when compared to that of the cytosol.

Some cells also export GSH [54]. The liver, for example, is the major organ for synthesis and export of GSH into the plasma [55]. Also, when cells or tissues are subjected to an oxidative stress, export of GSSG has been observed [56]. Oxidative stress results in the formation of GSSG at the expense of GSH. This shift in the ratio of [GSH]/[GSSG] would change the redox state to a more positive potential; if the potential rises too much this would clearly be detrimental. Export of GSSG would prevent this shift. The ability to export GSSG could be an important factor in the sensitivity of cells to oxidative stress. It has been shown that induction of oxidative stress by perfusion of isolated heart with *tert*-butylhydroperoxide results in the rapid efflux of GSSG [57,58]. This efflux appears to be a part of the protection of cells and tissues from oxidative stress. A potential reason for the efflux of GSSG may be to maintain the half-cell reduction potential of the GSSG/2GSH couple and a favorable redox environment in the cell. By removing GSSG, the denominator in the Nernst equation becomes smaller, thereby maintaining a more negative reduction potential.

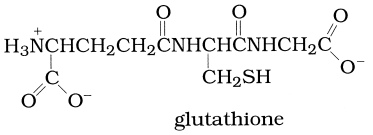
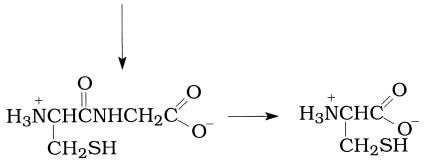
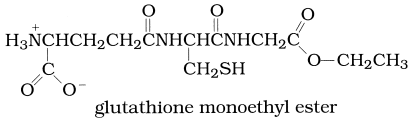
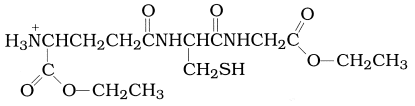
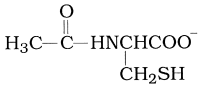
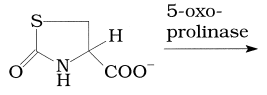
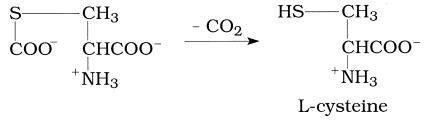
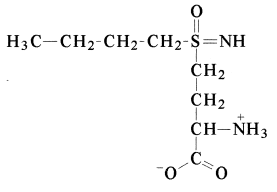
In both the research lab and the clinic there is often a need to alter the GSH pool. Many tools have been developed to modulate the cellular GSSG/2GSH redox system; examples are presented in Table 3 and Fig. 5.

A recent forum on glutathione in *Free Radical Biology and Medicine* provides more information on GSH and its cellular functions [59], regulation of GSH synthesis [60], gene expression and thiol redox state [52], BCL-2 and GSH [61], glutathione peroxidases [62], thiol-dependent hydroperoxide metabolism in parasites [63], and export pumps for GSH S-conjugates [64].

ROLE OF PROTEIN SULFHYDRYLS IN THE CELLULAR REDOX ENVIRONMENT

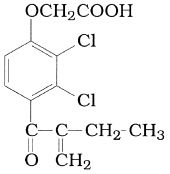
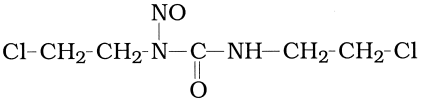
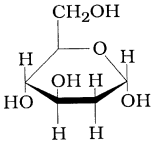
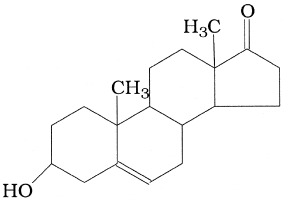
Numerous proteins contain sulfhydryl groups (PSH) due to their cysteine content. In fact, the concentration of PSH groups in cells and tissues is much greater than that of GSH [65]. These groups can be present as thiols (-SH), disulfides (PS-SP), or as mixed disulfides, for example, PS-SG when conjugated with GSH). Proteins can bind GSH, cysteine, homocysteine, and γ-glutamylcysteine to form mixed disulfides, but GSH is the dominant ligand [66]. The oxidation of the thiol form of an enzyme or the reduction of the disulfide form of an enzyme can result in activation or inactivation of enzyme function [67,68]. Protein S-thiolation-dethiolation is a dynamic process that occurs under physiological conditions in cells. This process is reversible and occurs at different rates depending on the protein and the nature of the thiol groups in the protein [69]. The protein S-glutathiolation/deglutathiolation status should, in some way, be a reflection of the redox state of the GSH-system in the cell. The oxidation of protein sulfhydryls to mixed disulfides is an early cellular response to oxidative stress

Table 3. Modulation of GSH and NADPH

Name	Structure	Function	Ref.
Tools to increase the GSH pool			
GSH, glutathione	 glutathione  cysteine-glycine cysteine	GSH is taken up only in small amounts by most cell types. Thus, feeding GSH to rodents increases their blood and urine levels but not their tissue GSH. Glutathione has to be broken down into its amino acids, transported into the cell and then resynthesized. Cysteine is not a good GSH source either, as it can be toxic.	[47,160–162]
GSH-ester	 glutathione monoethyl ester  glutathione diethyl ester	GSH-esters enter the cell and increase the GSH level. Monoethyl ester is hydrolyzed to GSH and ethanol. GSH diethyl ester transports more efficiently into cells where it is rapidly hydrolized to GSH monoethyl ester and then to GSH.	[163]
NAC, N-acetyl-L-cysteine, L-α-acetamido-β-mercapto-propionic acid		NAC is hydrolyzed to cysteine inside the cell. It is also an antioxidant and can scavenge various ROS and RNS such as hydroxyl radicals, hypochlorous acid, and peroxynitrite. It has limited toxicity and is used for treatment of various respiratory disorders and overdose of acetaminophen in humans.	[164]
OTC, L-2-oxo-thiazolidine 4-carboxylate	 L-2-oxothiazolidine-4-carboxylate  L-cysteine	OTC is a precursor for cysteine. It is transported into the cell and metabolized forming cysteine. It improves arterial dilation in patients with documented coronary artery disease, suggesting that intracellular GSH status is an important determinant of endothelial function.	[165,166,167]
Tools to decrease the GSH pool			
BSO, L-buthionine-SR-sulfoximine, Amino acid sulfoximines		BSO selectively inhibits γ-glutamyl-cysteinesynthetase (γ-GCS) thereby inhibiting the synthesis of γ-glutamylcysteine, a precursor of glutathione. BSO mainly decreases the cytosolic GSH. Although GSH synthesis is blocked, the cell's use of GSH, the export of GSSG, and the reduction of GSSG to GSH by glutathione reductase continues.	[43–45,168]

(Continued)

Table 3. *Continued*

Name	Structure	Function	Ref.
EA, Ethacrynic acid		Ethacrynic acid conjugates GSH via glutathione-S-transferase. EA prevents the use of GSH, but not its synthesis. EA reduces the cytosolic and mitochondrial GSH pool.	[169]
BCNU, Carmustine (1,3-*bis*(2-chloroethyl)-N-nitrosourea)		BCNU carbamoylates the lysine residue in the active site of glutathione reductase (GR). This NADPH-dependent reaction is fast and inhibits GR, thereby preventing the recycling of GSSG to GSH.	[170]
Tools to decrease NADPH			
2-Deoxy-D-glucose		2-Deoxy-D-glucose interferes with glucose utilization, reducing the formation of glucose-6-phosphate. Glucose-6-phosphate is the substrate for glucose-6-phosphate dehydrogenase, the enzyme that recycles $NADP^+$ to NADPH. Thus, 2-deoxy-D-glucose decreases the cellular NADPH pool and subsequently GSH.	[171,172]
DHEA, Dehydroepian drosterone, Dehydroiso androsterone		DHEA inhibits glucose-6-phosphate dehydrogenase thereby preventing the recycling of $NADP^+$ to NADPH.	[173]
Xenobiotics	Cytochrome P-450	Xenobiotics that increase the flux through the P-450 system	[174,175]

[70]. The formation of mixed disulfides can occur by several mechanisms:

Two-electron oxidation of a protein-thiol, followed by reaction with GSH

$$PSOH^{6} + GSH \rightarrow PS\text{-}SG + H_2O \qquad (24)$$

or one-electron oxidation of PSH or GSH, then formation of a disulfide

$$PS^{\bullet} + GS^{-} + O_2 \rightarrow PS\text{-}SG + O_2^{\bullet -} \qquad (25)$$

$$PS^{-} + GS^{\bullet} + O_2 \rightarrow PS\text{-}SG + O_2^{\bullet -} \qquad (26)$$

or by thiol, disulfide exchange reactions

$$PSH + GSSG \rightarrow PS\text{-}SG + GSH \qquad (27)$$

$$'PSH + PS\text{-}SG \rightarrow 'PS\text{-}SP + GSH \qquad (28)$$

$$PSH + 'PS\text{-}SG \rightarrow PS\text{-}SG + 'PSH \qquad (29)$$

These reactions demonstrate that PSH can play a role in the antioxidant network of cells and thereby influence the redox environment of the cell. It has been demonstrated that during the respiratory burst of neutrophils, up to 17% of the GSH can become protein-bound [66]. Reactions 27 and 28 however, restore GSH in the cell; reaction 27 not only restores GSH but removes GSSG. This will increase the value of the numerator and decrease the value of the denominator in the Nernst equation as written above (Eqn. 21), maintaining the reduction potential for the GSSG/2GSH half-cell. This reaction will preserve glutathione in the cell and serve as a buffer for the reduction potential. In contrast, the efflux of GSSG to maintain the redox status during oxidative stress will result in the loss of glutathione from the cell, thereby

[6]The formation of the sulfenic acid, PSOH, is a two-electron oxidation of a thiol, PSH. This intermediate is often written as PS^+.

decreasing the reducing capacity, which can only be replaced by synthesis of new GSH. Once the oxidative stress has been met and the restoration of an appropriate $[GSH]^2/[GSSG]$ ratio is underway, PSH can be returned to the cellular thiol pool by the reaction of GSH with PS-SG:

$$\text{PS-SG} + \text{GSH} \rightarrow \text{PSH} + \text{GSSG} \qquad (30)$$

GSH will be regenerated from GSSG through the glutathione reductase (GR) enzyme system:

$$\text{GSSG} + \text{NADPH} + \text{H}^+ \xrightarrow{\text{GR}} 2\text{GSH} + \text{NADP}^+ \qquad (31)$$

Dethiolation of proteins, reaction 30, also seems to be an enzyme-dependent process. Glutaredoxin, protein disulfide isomerase, and thioredoxin reductase are candidate enzymes to facilitate this reaction [66,71]. In a mixture of S-thiolated proteins from hepatocytes, glutaredoxin reduced the protein disulfides more effectively than the other two enzymes [72]. Thioredoxin was efficient only in combination with glutaredoxin, suggesting that it first reduces glutaredoxin, which then reduces the S-thiolated protein. On the other hand, thioredoxin has been shown to reduce glyceraldehyde-3-P-dehydrogenase in endothelial cells exposed to H_2O_2 [73]. Thus, glutaredoxin and thioredoxin may have different substrate preferences.

How well protein sulfhydryls serve as a redox buffer will depend on their reactivity with GSH. In erythrocytes it has been found that the reactivity of PSHs depends on the pK_a of the thiol and structural features, such as accessibility [69]. For example, in response to oxidative stress the β125-SH residue in rat hemoglobin is oxidized more rapidly than GSH, resulting in a mixed disulfide (PS-SG), reaction 24. Seres et al. found three different classes of protein-thiols in monocytes that varied in their levels of S-thiolation before, during, and after the respiratory burst [66]. These results show that S-glutathiolation of proteins is reversible after oxidative stress.

Because the redox environment of a cell depends both on its reduction potential and reducing capacity, protein sulfhydryls could be very important in maintaining the redox environment of a cell; PSH can serve as a buffer to maintain the reduction potential of the GSSG/2GSH half-cell; the large pool of PSH in cells will maintain the capacity of the GSH system to meet an oxidative stress. PSH might also be responsible for the lower ratio of GSH/GSSG in endoplasmic reticulum. Similar to the NADPH binding sites, there might be more PSH in the endoplasmic reticulum to bind GSH, shifting the ratio resulting in a more oxidizing potential. The importance of the protein thiol pool in the antioxidant network is an active area of investigation.

THE CELLULAR REDOX ENVIRONMENT THROUGHOUT THE LIFE OF A CELL

Two of the major pathways for signaling in cells involve: (i) phosphorylation of proteins, or (ii) changes in the thiol status of proteins due to changes in the redox environment of the cell. Both oxidative and reductive stress can trigger redox cascades that bring about changes in the thiol status of the cell. Changes in the cellular redox environment can alter signal transduction, DNA and RNA synthesis, protein synthesis, enzyme activation, and even regulation of the cell cycle [31,52, 74–82]. Activities such as ligand binding, DNA binding, and nuclear translocation have been shown to be under redox control [83,84]. Most eukaryotic transcription factors have been found to be active only in the reduced form. Translocation of transcription factors to the nucleus is often redox-dependent [85]. It has been shown that the nuclear translocation of the glucocorticoid receptor was inhibited in the presence of 0.5–2 mM H_2O_2 [86]. High concentrations of thiols have been shown to stimulate proliferation of some tumor cells [87–89], consistent with a more reducing environment being a factor for increased proliferation. In contrast, cell death is initiated by oxidizing environments. For example, pro-oxidants such as certain arachidonic acid metabolites [90], lipid hydroperoxides [91], redox cycling compounds such as bleomycin [92] and quinones [93], reactive aldehydes [94], and dithiocarbamates [95] increase the intracellular concentration of reactive oxygen species that can induce apoptosis. Antioxidants that serve as reducing agents, such as NAC, GSH, and thiol-containing proteins (e.g., thioredoxin), have been shown to prevent apoptosis [96–98]. Thus, the redox environment of the cell might determine if a cell will proliferate, differentiate, or die [30,99–104].

Proliferation and differentiation

Development of an advanced organism starts with the division of a single cell. The progeny of this cell must turn into a multi-cell, multi-tissue, and multi-functional entity. This development occurs in a relatively reducing environment. In prenatal tissue GSH levels are relatively high while antioxidant enzyme levels (SOD, GPx, and catalase) are low, consistent with a reducing environment [105–107]. During the last few days in utero, rabbit lung-SOD and catalase levels increase 2-fold; GPx increases 4-fold and GSH appears to decrease [108,109]. These changes appear to be preparations for higher oxygen levels to be encountered after birth. But they also suggest a general change to a more oxidized redox environment.

A clear example of the changes in redox environment

during differentiation is provided by Allen et al. in a study of a slime mold (*Physarum polycephalum*) [110]. A sequential change in the antioxidant profile was observed upon providing a stimulus for differentiation. First, a substantial decrease (75%) in GSH was observed followed by a 7-fold increase in MnSOD; this increase appeared before the progression to a differentiated form was observed morphologically. In sea urchin eggs, fluctuations of cellular thiols during development were also noted [111–114]. Rapkine found that trichloroaceticacid-soluble thiol groups decrease during the preparatory stages of the first division, then increase during formation of the mitotic apparatus [115]. Kawamura and Dan investigated the mitotic cycle in a sea urchin egg and found that fertilization increased protein sulfhydryl groups, resulting in high concentrations in prophase, metaphase, and anaphase, and then disappearing in telophase [116]. Sandritter and Krygier found in HeLa cells that protein-bound sulfhydryl groups increased in the pre-mitotic stage, decreased as the mitotic apparatus forms, and increased again at the end of cell division [117]. More recent data indicate that intracellular GSH levels fluctuate during the cell cycle [89,114]. Li and Oberley suggest that changes in the redox environment are necessary for a cell to successfully progress through the cell cycle [118]. They found that during the M phase of the cell cycle of NIH/3T3 cells, total glutathione and glutathione disulfide levels were greatly increased compared to quiescent cells and cells in S phase. Also, Atzori et al. have reported that after the seeding of cells for tissue culture experiments, GSH levels increase during the lag phase, are high during the initial exponential growth phase, and then fall as cells become confluent [119]. These reports are consistent with the redox environment of a cell having a significant role in proliferation.

Induction or inhibition of proliferation due to oxidative stress seems to be dependent on levels of oxidants in the cell. Low levels of oxidants appear to stimulate proliferation while higher levels inhibit [93, 120–123]. Oxidative stress might also select between cells needed for development and those that are redundant. Pierce et al. proposed that hydrogen peroxide in blastocele fluid induces apoptosis in redundant retrophectodermal inner cell mass cells. Cells with embryonic potential that will continue development into the embryo and placenta are protected against H_2O_2-induced apoptosis due to their glutathione content [124]. These observations are consistent with a more reducing environment being associated with proliferation [101,115,125,126] and a more oxidizing environment initiating differentiation [30,127].

Death

On the other end of life, oxidizing environments can result in apoptosis or necrosis. Apoptosis may occur with moderate, but lethal oxidative stimuli; whereas necrosis would result from severe oxidative challenges. This is logical because apoptosis requires energy in the form of ATP to carry out the organized program of cell death [128–132]. A severe oxidative stress would deplete energy stores and damage the machinery that produces the energy needed to implement the structural changes associated with apoptosis. For example, apoptosis in various cells is induced by exposure to low levels of H_2O_2; at higher levels necrosis is induced [133–135]. In leukemia cells 9–30 μM H_2O_2 induces apoptosis; concentrations $\geq$ 100 μM result in necrosis [136,137]. Cai and Jones found that apoptosis was associated with a change in reduction potential of +72 mV in HL-60 cells (i.e., E_{hc} increased from -239 ± 6 to -167 ± 9 mV) or a change of +86 mV in HT29 cells [102], Table 4. There are different stages in the apoptotic pathway:

1) initiation, where a signal is received;
2) the effector phase, where the signals are integrated and the decision to live or die is made; and
3) the irreversible execution phase with digestion of DNA and proteins.

It has been suggested that Bcl-2 could be the final determinant as to whether cells enter the execution phase or not [138]. Cytochrome c release precedes a decrease in glutathione [102]; Bcl-2 can block cytochrome c release. In fact, overexpression of Bcl-2 can make the cell more reducing without influencing overall antioxidant enzyme status.

Thus, cellular redox environment can be connected to Bcl-2 and appears to be the factor that brings about the transition from stage 2 to stage 3, as outlined above [61,139]. We suggest that the redox environment may be the final determinant for the execution of apoptosis. This is supported by the finding that a decrease in GSH levels correlates with apoptosis in lymphoid cells [140].

Necrosis is thought to occur at a more oxidizing redox environment. Because cells literally fall apart and both GSH and GSSG leak into the extracellular environment during necrosis, E_{hc} will increase. The actual cellular redox environment would be difficult to measure.

Thiols and disulfides as nano-switches

The reduction potential of various redox couples in the cell could be viewed as trigger to activate a cellular switchboard. By changing the reduction potential of re-

Table 4. Reduction Potential and Biological Status of Cells

Cell line	Treatment[a]	E_{hc}/mV for GSSG/2GSH (pH) Proliferating	Confluent	Differentiating	Apoptotic	Ref.
HL-60	1 μM staurosporine	-239 ± 6[b]			-167 ± 9[b]	[102]
HL-60	Overexpressing Bcl-2 + 1 μM staurosporine	-230[b] to -205[b]			no apoptosis at $E \leq -205$[b]	[102]
Normal fibroblast	Untreated	-222 (7.0)[c] -247 (7.4)	-188 (7.0)[c,d] -213 (7.4)			[103]
Fibrosarcoma	Untreated	-213 (7.0)[c] -238 (7.4)	-213 (7.0)[c,e] -238 (7.4)			[103]
HT29	5 mM sodium butyrate	-258 (7.39)[f]		-201 (7.40)[f]		[30]
HT29	25 μM benzyl-isothiocyanate	-244 (7.30)[f]		-160 (7.45)[f]		[30]
Murine hybridoma[g]		-235[b]			-170[b]	[30,176]
CRL-1606 murine hybridoma[g]	Untreated	-232 (7.0)[c] -257 (7.4)				[53]
Jurkat	Untreated	-240[b]				[177]
WAL-2A human lymphocyte	Untreated	-237[b]				[177]
WAL-2A human lymphocyte	ρ^0 (no mitDNA)	-233[b]				[177]

[a] Changing cells from proliferation to another biological state.
[b] The data were adjusted to the measured cellular pH, but the pH was not reported.
[c] This reported E_{hc} assumed pH = 7.0. The E_{hc} below is adjusted to pH 7.4 with Eqn. 14.
[d] These cells were contact-inhibited.
[e] These cells were not contact-inhibited, thus, they continue to proliferate.
[f] This pH was determined experimentally.
[g] These cells are a fusion product of a myeloma and a B lymphocyte.

dox couples, a series of nano-switches[7] are activated that move the cell from proliferation through various stages of differentiation and, when stressed or damaged in such a way that the redox environment cannot be maintained, into apoptosis. Necrosis is the complete loss of the ability to activate and/or respond to changes in these nano-switches.

We hypothesize that GSH plays an important role in controlling these nano-switches. As the most abundant redox buffer in the cell, GSH will control the status of the protein thiol/disulfide equilibrium and thereby drive the status of the switches as if they are rheostats. Figure 6 is a simplified schematic of how E_{hc} of the GSSG/2GSH couple and this cellular switchboard may connect to the biological status of cells. Individual molecules can have their switches either on or off. But, because a cell has an ensemble of such molecules, the change from one state to another will look much like a titration curve of a weak acid or base. During proliferation (A) the switch for differentiation is essentially off. A change in E_{hc} toward more positive values will result in a shift in the equilibrium from the thiol form to the disulfide form of enzymes and proteins. Once a certain E_{hc} is reached (B), differentiation is initiated and some cells will take this course. The more positive E_{hc}, the higher the number of cells that will differentiate. When the differentiation switch turns fully on (C), the proliferation switch is turned down and finally off (D). If E_{hc} becomes too positive, cell death switches are activated and apoptosis can be initiated (E). If E_{hc} is very positive, programmed cell death cannot be carried out and necrosis results (F). Note, if proliferating

[7] A nano-switch is a very small switch that operates on a nanometer scale. For example, the distance between two sulfhydryls in a protein with two intervening amino acids, such as in thioredoxin.

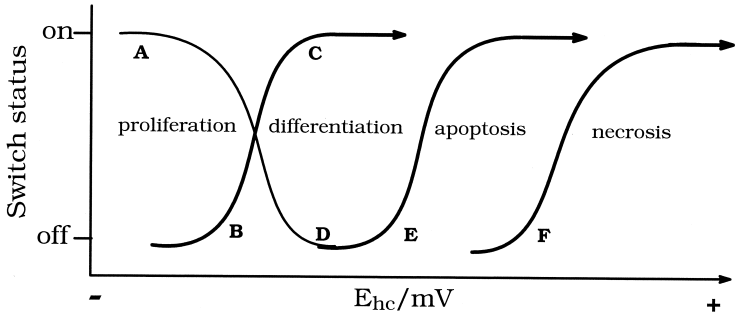


Fig. 6. Reduction potential-driven nano-switches move cells through different biological stages. The redox environment of a cell changes throughout its life cycle. During proliferation E_{hc} for the GSSG/2GSH couple has the most negative value. (A) The switches for proliferation are fully on. (B) When E_{hc} (GSH) becomes more positive, the differentiation switches can be turned on while proliferation decreases. (C) The more positive E_{hc} (GSH) becomes, the more differentiation switches are turned on until they reach a maximum where nearly all cells are differentiating. (D) While cells undergo differentiation, proliferation switches are turned down and finally turned off. Cells that are not terminally differentiated could undergo proliferation with an appropriate signal and associated redox environment. We would expect that these partially differentiated cells might shift to a more negative reduction potential, i.e., to the left. (E) If E_{hc} (GSH) becomes too positive, then death signals are activated and apoptosis is initiated. This mechanism provides for the orderly removal of cells that have lost their ability to control their redox environment and therefore, are not functioning normally. It should also coincide with signaling pathways to purposely dispose of unneeded cells. (F) Very high values of E_{hc} (GSH), resulting from severe oxidative stress, leave only necrosis as a path to cell death.

cells suffer a very large change in E_{hc}, they could be pushed from proliferation directly into the apoptotic or necrotic area.

In general there are two types of thiol/disulfide switches. The Type I switch is:

$$GSSG + PSH \rightleftharpoons PSSG + GSH \qquad \text{(Type I)} \tag{32}$$

The equilibrium constant for this switch will be:

$$K = \frac{[GSH]\,[PSSG]}{[GSSG]\,[PSH]} \tag{33}$$

For example, if [GSH] = 5 mM and [GSSG] = 25 μM, then using equation (21) $E_{hc} = -240$ mV. If [GSH] decreases to 2.5 mM and [GSSG] increases to 100 μM, a +35 mV change will occur resulting in $E_{hc} = -205$ mV (the redox environment associated with differentiation, Table 4). The [PSSG]/[PSH] ratio will change by a factor of 8, resulting in an 8-fold change in the status of the switch.

If the nano-switch is driven by a Type II reaction:

$$GSSG + P(SH)_2 \rightleftharpoons PSS + 2GSH \qquad \text{(Type II)} \tag{34}$$

then, the equilibrium constant will have the form:

$$K = \frac{[GSH]^2\,[PSS]}{[GSSG]\,[P(SH)_2]} \tag{35}$$

Using the example for the Type I switch, this same +35 mV change will result in a change of the [PSS]/[$P(SH)_2$] ratio by a factor of 16. Thus, a Type II switch translates changes in reduction potential into a 2-fold greater change in the status of the nano-switch, compared to a Type I switch.

As another example, if the difference in reduction potential for GSSG/2GSH for apoptotic vs. proliferating cells is +75 mV (Table 4), then from the Nernst equation, the change in this couple would be:

$$+75\ \text{mV} = -30.8\ \text{mV} \log [GSH]^2/[GSSG] \tag{36}$$

This +75 mV change corresponds to a 1/300 change in the $[GSH]^2/[GSSG]$ ratio. If the glutathione system is in equilibrium with a Type II switch, then a 300-fold change will occur in this nano-switch. This large change could shift cells from a proliferating state into apoptosis.

The GSSG/2GSH couple is clearly important in the control of the biological state of a cell, but it is not the sole determinant. Signaling events can bring about changes in metabolism that will change the $[GSH]^2/[GSSG]$ ratio. But this couple will initiate its own signals. The chemistry of the GSSG/2GSH couple could make it an important "effector" for determination of the biological state of a cell.

One must be mindful that oxidative stimuli need not always move a cell to a more oxidized biological state, for example, from proliferation to differentiation (to the right in Fig. 6). It has been shown that stimulation by mild oxidants can induce normal cells to divide. Oxidative stimuli seem to be normal regulators of cellular transcription. However, when the capacity to detoxify the oxidants produced is exceeded, then a series of events is triggered that can induce cell death [141,142]. For

example, increased hydrogen peroxide production in vascular smooth muscle cells is associated with increased proliferation [121]. Another example is the increased rate of proliferation observed by Ras-overexpressing cells [143]. This appears to be the result of increased production of superoxide and hydrogen peroxide. As first suggested by Oberley et al. [120], oxidative stimuli may result in signaling pathways leading to proliferation. The increase in oxidant production should not be interpreted as necessarily resulting in a more oxidative environment. Rather the cellular response may result in an overall more reducing environment because of the production of reducing equivalents in response to an oxidative stress.

Cancer can be viewed as a state where the balance between cell proliferation and cell death has shifted inappropriately toward excess proliferation, suggesting that cancer tissue would have a more reducing environment.

As emphasized above, the concentration of GSH is an important factor in determining the reduction potential for the GSSG/2GSH couple. Depending on the total concentration of GSH in a cell, the magnitude of an oxidative event associated with the initiation of differentiation or cell death will vary. As seen in Fig. 7, for the cell to change from proliferation through the full range of differentiation (−250 mV to −190 mV), the cell with 1 mM GSH needs to have only 18 μM GSH oxidized to GSSG, whereas a cell with 10 mM GSH will require that 380 μM GSH be oxidized. This clearly demonstrates the role of "reducing capacity" in maintaining the redox environment of the cell. This also explains, in part, why different cells can react quite differently to similar oxidative insults.

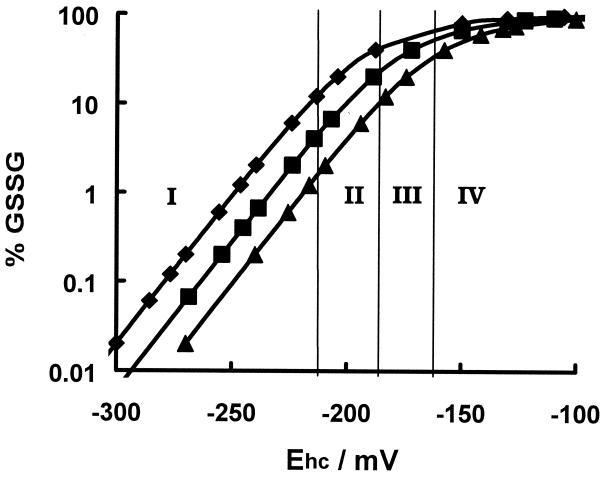


Fig. 7. Model of how E_{hc} for the GSSG/2GSH couple may be associated with the different physiological states and changes in a cell or tissue. The model is derived from Table 4; data points are derived from calculations using Eqn. 21. The proliferating state is shown with a reduction potential of the glutathione half-cell somewhere in the range of E_{hc} = −260 to −210 mV (I). Differentiation is associated with a more positive potential, −210 to −180 mV (II). If E_{hc} rises from −180 to −160 mV (III), then apoptosis is triggered. If E_{hc} rises above approximately −160 mV (IV), then necrosis will be the dominant route to cell death. The change-over from apoptosis to necrosis at around −160 mV was chosen on the basis of the shape of the curves shown in this figure. These curves show that at around −160 to −150 mV the redox-buffering capacity of the GSH system is essentially lost. If the cell is unable to restore the GSH redox buffer, it will not be able to produce the energy needed to repair the damage to continue living or even to carry out the apoptosis program. As seen in Table 4, few studies have made exact estimates of concentrations of GSH and GSSG, so exact borders between various states are as yet unknown. Symbols: ◆ = 10 mM GSH; ■ = 3 mM GSH; ▲ = 1 mM GSH; I = range of proliferation; II = range of differentiation; III = range of apoptosis; IV = range of necrosis.

SUMMARY

It is now realized that redox changes in the cell will initiate various signaling pathways [144–149]. Research in this area is in its infancy and is mostly observational, in that cells and tissues are subjected to an oxidative or reductive stress and the effects observed. The research community, in general, has not yet related the applied stress to quantitative changes in cellular redox environment or quantitative changes in the redox status of specific redox couples, but only qualitative changes. We do not know, on a quantitative basis, the "redox environment" and the redox state of specific couples needed to initiate a particular set of cellular signals. We need numerical indicators of the redox state of specific redox pairs as well as redox environment. In the next decades, research in this area will need to become more quantitative so maximal understanding of the ubiquitous term "redox state" can be achieved. This will help answer questions such as: when is the redox environment the cause, an effect or a co-conspirator in the biological events occurring in an organism?

Because the redox state for a redox pair depends on both the reduction potential and reducing capacity, both should be specified. A convenient notation for the status of a redox pair, such as GSSG/2GSH, would be {E_{hc} (GSH); [GSH]}, for example, {−180 mV (GSH); 3.5 mM}. Here −180 mV is the half-cell reduction potential of the couple of interest; (GSH) denotes the particular redox couple, and 3.5 mM is the concentration of the reduced species of that couple. This approach represents a first step into a new area of quantitative biology that will be of utmost importance in this new century of scientific endeavor. An understanding of the cellular redox environment will allow us to address issues in health and disease with the insight and rigor needed for the advances we want to make.

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REFERENCES

[1] Bücher, T.; Klingenberg, M. Wege des Wasserstoffs in der lebendigen Organisation. *Angew. Chem.* **70:**552–570; 1958.

[2] Krebs, H. A. The redox state of nicotinamide adenine dinucleotide in the cytoplasm and mitochondria of rat liver. *Adv. Enzyme Regul.* **5:**409–434; 1967.

[3] Krebs, H. A.; Gascoyne, T. The redox state of the nicotinamide-adenine dinucleotides in rat liver homogenates. *Biochem. J.* **108:**513–520; 1968.

[4] Krebs, H. A.; Veech, R. L. Equilibrium relations between pyridine nucleotides and adenine nucleotides and their roles in the regulation of metabolic processes. *Adv. Enzyme Regul.* **7:**397–413; 1969.

[5] Wardman, P. Reduction potentials of one-electron couples involving free radicals in aqueous solution. *J. Phys. Chem. Ref. Data* **18:**1637–1755; 1989.

[6] Sies, H. Nicotinamide nucleotide compartmentation. In: Sies, H., ed. *Metabolic compartmentation*. London: Academic Press; 1982:205–231.

[7] Buettner, G. R. The pecking order of free radicals and antioxidants: Lipid peroxidation, α-tocopherol, and ascorbate. *Arch. Biochem. Biophys.* **300:**535–543; 1993.

[8] Koppenol, W. H. The chemical reactivity of radicals. In: Wallace, K. B., ed. *Free radical toxicology*. London: Taylor and Francis; 1997:3–14.

[9] Koppenol, W. H.; Bulter, J. Energetics of interconversion reactions of oxyradicals. *Adv. Free Radic. Biol. Med.* **1:**91–131; 1985.

[10] Turrens, J. F.; Alexandre, A.; Lehninger, A. L. Ubisemiquinone is the electron donor for superoxide formation by complex III of heart mitochondria. *Arch. Biochem. Biophys.* **237:**408–414; 1985.

[11] Beyer, R. E. The participation of coenzyme Q in free radical production and antioxidation. *Free Radic. Biol. Med.* **8:**545–565; 1990.

[12] Nohl, H.; Gille, L.; Kozlov, A.; Staniek, K. The role of ubiquinone in biology and medicine. *Curr. Top. Biophys.* **22:**158–164; 1988.

[13] Forman, H. J.; Kennedy, J. Superoxide production and electron transport in mitochondrial oxidation of dihydroorotic acid. *J. Biol. Chem.* **250:**4322–4326; 1975.

[14] Wenk, J.; Brenneisen, P.; Wlaschek, M.; Poswig, A.; Briviba, K.; Oberley, T. D.; Scharffetter-Kochanek, K. Stable overexpression of manganese superoxide dismutase in mitochondria identifies hydrogen peroxide as a major oxidant in the AP-1-mediated induction of matrix-degrading metalloprotease-1. *J. Biol. Chem.* **274:**25869–25876; 1999.

[15] Li, S.; Yan, T.; Qin, Y.; Oberley, T. D.; Oberley, L. W. The role of cellular glutathione peroxidase redox regulation in the suppression of tumor cell growth by manganese superoxide dismutase. *Cancer Res.* **60:**3927–3939; 2000.

[16] Clark, W. M. *Oxidation-reduction potentials of organic systems*. Baltimore, MD: Williams & Wilkins; 1960.

[17] Lardy, H. A. *Respiratory enzymes*. Minneapolis, MN: Burgess; 1949.

[18] Burton, K. Free energy data of biological interest. *Ergeb. Physiol.* **49:**275; 1957.

[19] Jensen, M. A.; Elving, P. J. Nicotinamide adenine dinucleotide (NAD^+) formal potential of the $NAD^+/NAD^\bullet$ couple and $NAD^\bullet$ dimerization rate. *Biochim. Biophys. Acta* **764:**310–315; 1984.

[20] Rodkey, F. L. Oxidation-reduction potentials of the triphosphopyridine nucleotide system. *J. Biol. Chem.* **213:**777–786; 1955.

[21] Land, E. J.; Swallow, A. J. One-electron reactions in biochemical systems as studied by pulse radiolysis. IV. Oxidation of dihydronicotinamide-adenine dinucleotide. *Biochim. Biophys. Acta* **234:**34–42; 1971.

[22] Reich, J. G.; Sel'kov, E. E. Energy metabolism of the cell: a theoretical treatise. New York: Academic Press; 1981.

[23] Bücher, T.; Brauser, B.; Conze, A.; Klein, F.; Langghuth, O.; Sies, H. State of oxidation-reduction and state of binding in the cytosolic NADH-system as disclosed by equilibration with extracellular lactate/pyruvate in hemoglobin-free perfused rat liver. *Eur. J. Biochem.* **27:**301–317; 1972.

[24] Bücher, T.; Sies, H. Metabolic interaction of mitochondrial and cytosolic systems in rat liver. In: Nover, L.; Lynen, F.; Mothes, K., eds. *Cell compartmentation and metabolic channeling*. Jena, Germany: Fischer Verlag; 1980:279–302.

[25] Veech, R. L.; Eggleston, L. V.; Krebs, H. A. The redox state of free nicotinamide-adenine dinuleotide phosphate in the cytoplasm of rat liver. *Biochem. J.* **155:**609–619; 1969.

[26] Krebs, H. A.; Veech, R. L. Pyridine nucleotide interrelations. In: Papa, S.; Tager, J. M.; Slater, S. C., eds. *The energy level and metabolic control in mitochondria*. Bari: Adriatica Editrice; 1969:329–382.

[27] Moyle, J.; Mitchell, P. The proton-translocating nicotinamide-adenine dinucleotide (phosphate) transhydrogenase of rat liver mitochodnria. *Biochem. J.* **132:**571–585; 1973.

[28] Gilbert, H. F. Molecular and cellular aspects of thiol-disulfide exchange. In: Meister, A., ed. *Advances in enzymology*. New York: Wiley Interscience; 1990:69–173.

[29] Smith, C. V.; Jones, D. P.; Guenthner, T. M.; Lash, L. H.; Lauterburg, B. H. Contemporary issues in toxicology. Compartmentation of glutathione: implications for the study of toxicity and disease. *Toxicol. Appl. Pharmacol.* **140:**1–12; 1996.

[30] Kirlin, W. G.; Cai, J.; Thompson, S. A.; Diaz, D.; Kavanagh, T. J.; Jones, D. P. Glutathione redox potential in response to differentiation and enzyme inducers. *Free Radic. Biol. Med.* **27:**1208–1218; 1999.

[31] Matthews, J. R.; Wakasugi, N.; Virelizier, J. L.; Yodoi, J.; Hay, R. T. Thioredoxin regulates the DNA binding activity of NF-kappaB by reduction of a disulfide bond involving cysteine 62. *Nucleic Acid Res.* **20:**3821–3830; 1992.

[32] Okamoto, T.; Ogiwara, H.; Hayashi, T.; Mitsui, A.; Kawabe, T.; Yodoi, J. Human thioredoxin/adult T cell leukemia-derived factor activates the enhancer binding protein of human immunodeficiency virus type 1 by thiol redox control mechanism. *Int. Immunol.* **4:**811–819; 1992.

[33] Holmgren, A.; Luthman, M. Tissue distribution and subcellular localization of bovine thioredoxin determined by radioimmunoassay. *Biochemistry* **17:**4071–4077; 1978.

[34] Holmgren, A.; Ohlsson, I.; Grankvist, M. L. Thioredoxin from *Escherichia coli*. Radioimmunological and enzymatic determinations in wild type cells and mutants defective in phage T7 DNA replication. *J. Biol. Chem.* **253:**430–436; 1978.

[35] Sen, C. K. Redox signaling and the emerging therapeutic potential of thiol antioxidants. *Biochem. Pharmacol.* **55:**1747–1758; 1998.

[36] Luthman, M.; Holmgren, A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry* **21:**6628–6633; 1982.

[37] Lee, S. R.; Bar-Noy, S.; Kwon, J.; Levine, R. L.; Stadtman, T. C.; Rhee, S. G. Mammalian thioredoxin reductase: oxidation of the C-terminal cysteine/selenocysteine active site forms a thioselenide, and replacement of selenium with sulfur markedly reduces catalytic activity. *Proc. Natl. Acad. Sci. USA* **97:**2521–2526; 2000.

[38] Follmann, H.; Haberlein, I. Thioredoxins: universal, yet specific thiol-disulfide redox cofactors. *Biofactors* **5:**147–156; 1995–96.

[39] Aslund, F.; Berndt, K. D.; Holmgren, A. Redox potentials of glutaredoxins and other thiol-disulfide oxidoreductases of the thioredoxin superfamily determined by direct protein-protein redox equilibria. *J. Biol. Chem.* **272:**30780–30786; 1997.

[40] Karp, G. *Cell biology* (2nd ed.). New York: McGraw-Hill; 1984.

[41] Copenhaver, W. M.; Bunge, R. P.; Bunge, M. B. *Bailey's textbook of histology* (16th ed.). Baltimore: Williams & Wilkins; 1971.

[42] Ross, M. H.; Reith, E. J.; Romrell, L. J. *Histology, a text and atlas* (2nd ed.). Baltimore, MD: Williams & Wilkins; 1989.

[43] Griffith, O. W. Mechanism of action, metabolism, and toxicity of buthionine sulfoximine and its higher homologs, potent inhibi-

tors of glutathione synthesis. *J. Biol. Chem.* **257:**13704–13712; 1982.
[44] Griffith, O. W.; Meister, A. Differential inhibition of glutamine and γ-glutamylcysteine synthetase by α-alkyl analogs of methionine sulfoximine that induce convulsions. *J. Biol. Chem.* **253:** 2333–2338; 1978.
[45] Griffith, O. W.; Anderson, M. E.; Meister, A. Inhibition of glutathione biosynthesis by prothionine sulfoximine (S-n-propyl-homoxysteine sulfoximine), a selective inhibitor of γ-glutamylcysteine synthetase. *J. Biol. Chem.* **254:**1205–1210; 1979.
[46] Fernandez-Checa, J. C.; Kaplowitz, N.; Garcia-Ruiz, C.; Colell, A. Mitochondrial glutathione: importance and transport. *Semin. Liver Dis.* **18:**389–401; 1998.
[47] Griffith, O. W.; Meister, A. Origin and turnover of mitochondrial glutathione. *Proc. Natl. Acad. Sci. USA* **82:**4668–4672; 1985.
[48] Schnellmann, R. G.; Gilchrist, S. M.; Mandel, L. J. Intracellular distribution and depletion of glutathione in rabbit renal proximal tubules. *Kidney Int.* **34:**229–233; 1988.
[49] Bellomo, G.; Vairetti, M.; Stivala, L.; Mirabelli, F.; Richelmi, P.; Orrenius, S. Demonstration of nuclear compartmentalization of glutathione in hepatocytes. *Proc. Natl. Acad. Sci. USA* **89:** 4412–4416; 1992.
[50] Edgren, M. R.; Revesz, L. Compartmentalized depletion of glutathione in cells treated with buthionine sulfoximine. *Br. J. Radiol.* **60:**723–724; 1987.
[51] Jevtovic-Todorovic, V.; Guenthner, T. M. Depletion of a discrete nuclear glutathione pool by oxidative stress, but not by buthionine sulfoximine. *Biochem. Pharmacol.* **44:**1383–1393; 1992.
[52] Arrigo, A. P. Gene expression and the thiol redox state. *Free Radic. Biol. Med.* **27:**936–944; 1999.
[53] Hwang, C.; Sinskey, A. J.; Lodish, H. F. Oxidized redox state of glutathione in the endoplasmic reticulum. *Science* **257:**1496–1502; 1992.
[54] Lu, S. C. Regulation of hepatic glutathione synthesis: current concepts and controversies. *FASEB J.* **13:**1169–1183; 1999.
[55] Bray, T. M.; Taylor, C. G. Tissue glutathione, nutrition, and oxidative stress. *Can. J. Physiol. Pharmacol.* **71:**746–751; 1993.
[56] Sies, H.; Akerboom, T. P. M. Glutathione disulfide (GSSG) efflux from cells and tissues. *Methods Enzymol.* **105:**445–451; 1984.
[57] Ishikawa, T.; Sies, H. Cardiac transport of glutathione disulfide and S-conjugate: studies with isolated perfused rate heart during hydroperoxide metabolism. *J. Biol. Chem.* **259:**3838–3843; 1984.
[58] Ishikawa, T.; Zimmer, M.; Sies, H. Energy-linked cardiac transport system for glutathione disulfide. *FEBS Lett.* **200:**128–132; 1986.
[59] Sies, H. Glutathione and its role in cellular functions. *Free Radic. Biol. Med.* **27:**916–921; 1999.
[60] Griffith, O. W. Biologic and pharmacologic regulation of mammalian glutathione synthesis. *Free Radic. Biol. Med.* **27:**922–935; 1999.
[61] Voehringer, D. W. BCL-2 and glutathione: alterations in cellular redox state that regulate apoptosis sensitivity. *Free Radic. Biol. Med.* **27:**945–950; 1999.
[62] Brigelius-Flohe, R. Tissue-specific functions of individual glutathione peroxidases. *Free Radic. Biol. Med.* **27:**951–965; 1999.
[63] Flohe, L.; Hecht, H. J.; Steinert, P. Glutathione and trypanothione in parasitic hydroperoxide metabolism. *Free Radic. Biol. Med.* **27:**966–984; 1999.
[64] Keppler, D. Export pumps for glutathione S-conjugates. *Free Radic. Biol. Med.* **27:**985–991; 1999.
[65] Torchinsky, Y. M. *Sulphur in proteins*. Oxford, UK: Pergamon; 1981.
[66] Seres, T.; Ravichandran, V.; Moriguchi, T.; Rokutan, K.; Thomas, J. A.; Johnston, R. B. Protein S-thiolation and dethiolation during respiratory burst in human monocytes. *J. Immunol.* **156:**1973–1980; 1996.
[67] Watanabe, A.; Tabeta, K.; Kosaka, K. Glutathione-dependent interconversion of microheterogeneous forms of glucose-6-phosphate dehydrogenase in rat liver. *J. Biochem.* (Tokyo) **72:** 695–701; 1972.
[68] Ernest, M. J.; Kim, K. H. Regulation of rat liver glycogen synthetase. Reversible inactivation of glycogen synthetase D by sulfhydryl-disulfide exchange. *J. Biol. Chem.* **248:**1550–1555; 1973.
[69] Di Simplicio, P.; Cacace, M. G.; Lusini, L.; Giannnerini, F.; Giustarini, D.; Rossi, R. Role of protein -SH groups in redox homeostatis—the erythrocyte as a model system. *Arch. Biochem. Biophys.* **355:**145–152; 1998.
[70] Thomas, J. A.; Poland, B.; Honzatko, R. Protein sulfhydryls and their role in the antioxidant function of protein S-thiolation. *Arch. Biochem. Biophys.* **319:**1–9; 1995.
[71] Mustacich, D.; Powis, G. Thioredoxin reductase. *Biochem. J.* **346:**1–8; 2000.
[72] Jung, C. H.; Thomas, J. A. S-glutathiolated hepatocyte proteins and insulin disulfides as substrates for reduction by glutaredoxin, thioredoxin, protein disulfide isomerase, and glutathione. *Arch. Biochem. Biophys.* **335:**61–72; 1996.
[73] Schuppe-Koistinen, I.; Gerdes, R.; Moldeus, P.; Cotgreave, I. A. Studies on the reversibility of protein S-thiolation in human endothelial cells. *Arch. Biochem. Biophys.* **315:**226–234; 1994.
[74] Suzuki, Y. J.; Forman, H. J.; Sevanian, A. Oxidants as stimulators of signal transduction. *Free Radic. Biol. Med.* **22:**269–285; 1997.
[75] Nakamura, H.; Nakamura, K.; Yodoi, J. Redox regulation of cellular activation. *Annu. Rev. Immunol.* **15:**351–369; 1997.
[76] Powis, G.; Gasdaska, J. R.; Baker, A. Redox signaling and the control of cell growth and death. *Adv. Pharmacol.* **38:**329–359; 1997.
[77] Zehavi-Willner, T.; Kosower, E. M.; Hunt, T.; Kosower, N. S. Glutathione, V. The effects of the thiol-oxidizing agent diamide on initiation and translation in rabbit reticulocytes. *Biochim. Biophys. Acta* **228:**245–251; 1971.
[78] Holmgren, A. Hydrogen donor system for *Escherichia coli* ribonucleoside-diphosphate reductase dependent upon glutathione. *Proc. Natl. Acad. Sci. USA* **73:**2275–2279; 1976.
[79] Abate, C.; Patel, L.; Rauscher, F. J.; Curran, T. Redox regulation of fos and jun DNA-binding activity in vitro. *Science* **249:**1157–1161; 1990.
[80] Sen, C. K.; Packer, L. Antioxidant and redox regulation of gene transcription. *FASEB J.* **10:**709–720; 1996.
[81] Rahman, I.; MacNee, W. Regulation of redox glutathione levels and gene transcription in lung inflammation: therapeutic approaches. *Free Radic. Biol. Med.* **28:**1405–1420; 2000.
[82] Shackelford, R. E.; Kaufmann, W. K.; Paules, R. S. Oxidative stress and cell cycle checkpoint function. *Free Radic. Biol. Med.* **28:**1387–1404; 2000.
[83] Simons, S. S.; Pratt, W. B. Glucocorticoid receptor thiols and steroid-binding activity. *Methods Enzymol.* **251:**406–422; 1995.
[84] Makino, Y.; Yoshikawa, N.; Okamoto, K.; Hirota, K.; Yodoi, J.; Makino, I.; Tanaka, H. Direct association with thioredoxin allows redox regulation of glucocorticoid receptor function. *J. Biol. Chem.* **274:**3182–3188; 1999.
[85] Okamoto, K.; Tanaka, H.; Ogawa, H.; Makino, Y.; Eguchi, H.; Hayashi, S.; Yoshikawa, N.; Poellinger, L.; Umesono, K.; Makino, I. Redox-dependent regulation of nuclear import of the glucocorticoid receptor. *J. Biol. Chem.* **274:**10363–10371; 1999.
[86] Bresnick, E. H.; Sanchez, E. R.; Harrison, R. W.; Pratt, W. B. Hydrogen peroxide stabilizes the steroid-binding state of rat liver glucocorticoid receptors by promoting disulfide bond formation. *Biochemistry* **27:**2866–2872; 1988.
[87] Broome, J. D.; Jeng, M. W. Promotion of replication in lymphoid cells by specific thiols and disulfides in vitro. *J. Exp. Med.* **138:**574–592; 1973.
[88] Hewllet, G.; Opitz, H. G.; Schlumberger, H. D.; Lemke, H. Growth regulation of a murine lymphoma cell line by a 2-mercaptoethanol or macrophage-activated serum factor. *Eur. J. Immunol.* **7:**781–785; 1977.
[89] Hamilos, D. L.; Zelarney, P.; Mascali, J. J. Lymphocyte proliferation in glutathione-depleted lymphocytes: direct relationship

between glutathione availability and the proliferative response. *Immunopharmacology* **18:**223–235; 1989.

[90] Kim, H. S.; Lee, J. H.; Kim, I. K. Intracellular glutathione level modulates the induction of apoptosis by delta 12-prostaglandin J2. *Prostaglandins* **51:**413–425; 1996.

[91] Aoshima, H.; Satoh, T.; Sakai, N.; Yamada, M.; Enokido, Y.; Ikeuchi, T.; Hatanaka, H. Generation of free radicals during lipid hydroperoxide-triggered apoptosis in PC12h cells. *Biochim. Biophys. Acta* **1345:**35–42; 1997.

[92] Hamilton, R. F.; Li, L.; Felder, T. B.; Holian, O. A. Bleomycin induces apoptosis in human alveolar macrophages. *Am. J. Physiol.* **269:**L318–L325; 1995.

[93] Dypbukt, J. M.; Ankarcrona, M.; Burkitt, M.; Sjoholm, A.; Strom, K.; Orrenius, S.; Nicotera, P. Different prooxidant levels stimulate growth, trigger apoptosis, or produce necrosis of insulin-secreting RINm5F cells. The role of intracellular polyamines. *J. Biol. Chem.* **269:**30553–30560; 1994.

[94] Okado, A.; Kawasaki, Y.; Hasuike, Y.; Takahashi, M.; Teshima, T.; Fujii, J.; Taniguchi, N. Induction of apoptotic cell death by methylglyoxal and 3-deoxyglucosone in macrophage-derived cell lines. *Biochem. Biophys. Res. Commun.* **225:**219–224; 1996.

[95] Nobel, C. I.; Kimland, M.; Lind, B.; Orrenius, S.; Slater, A. F. Dithiocarbamates induce apoptosis in thymocytes by raising the intracellular level of redox-active copper. *J. Biol. Chem.* **270:** 26202–26208; 1995.

[96] Sandstrom, P. A.; Mannie, M. D.; Buttke, T. M. Inhibition of activation-induced death in T cell hybridomas by thiol antioxidants: oxidative stress as a mediator of apoptosis. *J. Leukoc. Biol.* **55:**221–226; 1994.

[97] Gabby, M.; Tauber, M.; Porat, S.; Simantov, R. Selective role of glutathione in protecting human neuronal cells from dopamine-induced apoptosis. *Neuropharmacology* **35:**571–578; 1996.

[98] Iwata, S.; Hori, T.; Sato, N.; Hirota, K.; Sasada, T.; Mitsui, A.; Hirakawa, T.; Yodoi, J. Adult T cell leukemia (ATL)-derived factor/human thioredoxin prevents apoptosis of lymphoid cells induced by L-cystine and glutathione depletion: possible involvement of thiol-mediated redox regulation in apoptosis caused by pro-oxidant state. *J. Immunol.* **158:**3108–3117; 1997.

[99] Flores, S. C.; McCord, J. M. Redox regulation by HIV-1 Tat transcription factor. In: Scandalios, J. G., ed. *Oxidative stress and the molecular biology of antioxidant defenses*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press; 1997:117–138.

[100] Aw, T. Y. Molecular and cellular responses to oxidative stress and changes in oxidation-reduction imbalance in the intestine. *Am. J. Clin. Nutr.* **70:**557–565; 1999.

[101] Burdon, R. H. Superoxide and hydrogen peroxide in relation to mammalian cell proliferation. *Free Radic. Biol. Med.* **18:**775–794; 1995.

[102] Cai, J.; Jones, D. P. Superoxide in apoptosis: mitochondrial generation triggered by cytochrome c loss. *J. Biol. Chem.* **273:** 11401–11404; 1998.

[103] Hutter, D. E.; Till, B. G.; Greene, J. J. Redox state changes in density-dependent regulation of proliferation. *Exp. Cell Res.* **232:**435–438; 1997.

[104] Jones, D. P. *What does it really mean to ask—what is the redox state of a cell, tissue or organism*? A presentation in the Sunrise Free Radical School, Oxygen Society/Free Radical Research Society Annual Meeting, Washington, DC; 1998.

[105] Allen, R. G.; Venkatraj, V. S. Oxidants and antioxidants in development and differentiation. *J. Nutr.* **122:**631–635; 1992.

[106] Sohal, R. S.; Allen, R. G. Oxidative stress as a causal factor in differentiation and aging: a unifying hypothesis. *Exp. Gerontol.* **25:**499–522; 1990.

[107] Allen, R. G.; Balin, A. K. Oxidative influence on development and differentiation: an overview of a free radical theory of development. *Free Radic. Biol. Med.* **6:**631–661; 1989.

[108] Frank, L.; Groseclose, E. E. Preparation for birth into an O_2-rich environment: the antioxidant enzymes in the developing rabbit lung. *Pediatr. Res.* **18:**240–244; 1984.

[109] Frank, L. Oxygen toxicity in eukaryotes. In: Oberley, L. W., ed. *Superoxide dismutase, vol. III*. Boca Raton, FL: CRC Press; 1985:1–44.

[110] Allen, R. G.; Newton, R. K.; Sohal, R. S.; Shipley, G. L.; Nations, C. Alterations in superoxide dismutase, glutathione, and peroxides in the plasmodial slime mold *Physarum polycephalum* during differentiation. *J. Cell Physiol.* **125:**413–419; 1985.

[111] Hammett, F. S. Cell division and cell growth in size. *Protoplasma* **7:**535–540; 1929.

[112] Kawamura, N. Cytochemical and quantitative study of protein-bound sulfhydryl and disulfide groups in eggs of *Arbacia* during the first cleavage. *Exp. Cell Res.* **20:**127–138; 1960.

[113] Mazia, D. Mitosis and the physiology of cell division. In: Brachet, J.; Mirsky, A. E. *The cell: biochemistry, physiology, morphology*. New York: Academic Press; 1961:250–257.

[114] Shaw, J. P.; Chou, I. N. Elevation of intracellular glutathione content associated with mitogenic stimulation of quiescent fibroblasts. *J. Cell. Physiol.* **129:**193–198; 1986.

[115] Rapkine, L. Sur les processus chimiques au cours de la division cellulaire. *Ann. Physiol. Physiochem. Biol.* **7:**382–418; 1931.

[116] Kawamura, N.; Dan, K. A cytochemical study of the sulfhydryl groups of sea urchin eggs during first cleavage. *J. Biophys. Biochem. Cytol.* **4:**615–619; 1958.

[117] Sandritter, W.; Krygier, A. Cytophotometrische Bestimmung von proteingebundenen Thiolen in der Mitose und Interphase von HeLa-Zellen. *Z. Krebsforsch.* **62:**596–610; 1959.

[118] Li, N.; Oberley, T. D. Modulation of antioxidant enzymes, reactive oxygen species, and glutathione levels in manganese superoxide dismutase-overexpressing NIH/3T3 fibroblast during the cell cycle. *J. Cell. Physiol.* **177:**148–160; 1998.

[119] Atzori, L.; Dypbukt, J. M.; Sundqvist, K.; Cotgreave, I.; Edman, C. C.; Moldeus, P.; Grafstrom, R. C. Growth-associated modifications of low-molecular-weight thiols and protein sulfhydryls in human bronchial fibroblasts. *J. Cell. Physiol.* **143:**165–171; 1990.

[120] Oberley, L. W.; Oberley, T. D.; Buettner, G. R. Cell division in normal and transformed cells: the possible role of superoxide and hydrogen peroxide. *Med. Hypotheses* **7:**21–42; 1981.

[121] Brown, M. R.; Miller, F. J.; Li, W. G.; Ellingson, A. N.; Mozena, J. D.; Chatterjee, P.; Engelhardt, J. F.; Zwacka, R. M.; Oberley, L. W.; Fang, X.; Spector, A. A.; Weintraub, N. L. Overexpression of human catalase inhibits proliferation and promotes apoptosis in vascular smooth muscle cells. *Circ. Res.* **85:**524–533; 1999.

[122] Yang, M.; Nazhat, N. B.; Jiang, X.; Kelsey, S. M.; Blake, D. R.; Newland, A. C.; Morris, C. J. Adriamycin stimulates proliferation of human lymphoblastic leukaemic cells via a mechanism of hydrogen peroxide (H_2O_2) production. *Br. J. Haematol.* **95:** 339–344; 1996.

[123] Burdon, R. H.; Rice-Evans, C. Free radicals and the regulation of mammalian cell proliferation. *Free Radic. Res. Commun.* **6:**345–358; 1989.

[124] Pierce, G. B.; Parchment, R. E.; Lewellyn, A. L. Hydrogen peroxide as a mediator of programmed cell death in the blastocyst. *Differentiation* **46:**181–186; 1991.

[125] Romero, F. J.; Zukowski, D.; Mueller-Klieser, W. Glutathione content of V79 cells in two- or three-dimensional culture. *Am. J. Physiol.* **272:**C1507–C1512; 1997.

[126] Post, G. B.; Keller, D. A.; Connor, K. A.; Menzel, D. B. Effects of culture conditions on glutathione content in A549 cells. *Biochem. Biophys. Res. Commun.* **114:**737–742; 1983.

[127] Alaluf, S.; Muir-Howie, H.; Hu, H. L.; Evans, A.; Green, M. R. Atmospheric oxygen accelerates the induction of a post-mitotic phenotype in human dermal fibroblasts: the key protective role of glutathione. *Differentiation* **66:**147–155; 2000.

[128] Liu, X.; Kim, C. N.; Yang, J.; Jemmerson, R.; Wang, X. Induction of apoptotic program in cell-free extracts: requirement for dATP and cytochrome c. *Cell* **86:**147–157; 1996.

[129] Richter, C.; Schweizer, M.; Cossarizza, A.; Franceschi, C. Control of apoptosis by cellular ATP level. *FEBS Lett.* **378:**107–110; 1996.

[130] Searle, J.; Kerr, F. J.; Bishop, C. J. Necrosis and apoptosis: distinct modes of cell death with fundamentally different significance. *Pathol. Annu.* **17:**229–259; 1982.
[131] Lee, Y.; Shacter, E. Oxidative stress inhibits apoptosis in human lymphoma cells. *J. Biol. Chem.* **274:**19792–19798; 1999.
[132] Lelli, J. L.; Becks, L. L.; Dabrowska, M. I.; Hinshaw, D. B. ATP converts necrosis to apoptosis in oxidant-injured endothelial cells. *Free Radic. Biol. Med.* **25:**694–702; 1998.
[133] Lennon, S. V.; Martin, S. J.; Cotter, T. G. Dose-dependent induction of apoptosis in human tumour cell lines by widely diverging stimuli. *Cell Prolif.* **24:**203–214; 1991.
[134] Buttke, T. M.; Sandstrom, P. A. Oxidative stress as a mediator of apoptosis. *Immunol. Today* **17:**7–10; 1994.
[135] Lee, Y. J.; Shacter, E. Bcl-2 does not protect Burkitt's lymphoma cells from oxidant-induced cell death. *Blood* **89:**4480–4492; 1997.
[136] Wagner, B. A.; Buettner, G. R.; Oberley, L. W.; Darby, C. J.; Burns, C. P. Myeloperoxidase is involved in H_2O_2-induced apoptosis of HL-60 human leukemia cells. *J. Biol. Chem.* **275:**22461–22469; 2000.
[137] Schafer, F.; Wagner, B. A.; Wang, H. P.; Oberley, L. W.; Buettner, G. R. Does peroxide toxicity correlate with antioxidant enzyme activity or oxidizability of cells? *Free Radic. Biol. Med.* **27:**S115; 1999.
[138] Eastman, A. The mechanism of action of cisplatin: from adducts to apoptosis. In: Lippert, B., ed. *Cisplatin: chemistry and biochemistry of a leading anticancer drug*. Zurich, Switzerland: Wiley-VCH; 1999:111–134.
[139] Ellerby, L. M.; Ellerby, H. M.; Park, S. M.; Holleran, A. L.; Murphy, A. N.; Fiskum, G.; Kane, D. J.; Testa, M. P.; Kayalar, C.; Bredesen, D. E. Shift of the cellular oxidation-reduction potential in neural cells expressing Bcl-2. *J. Neurochem.* **67:**1259–1267; 1996.
[140] Fernandes, R. S.; Cotter, T. G. Apoptosis or necrosis: intracellular levels of glutathione influence mode of cell death. *Biochem. Pharmacol.* **48:**675–681; 1994.
[141] Burdon, R. H.; Alliangana, D.; Gill, V. Endogenously generated active oxygen species and cellular glutathione levels in relation to BHK-21 cell proliferation. *Free Radic. Res.* **21:**121–133; 1994.
[142] Nishioka, W. K.; Welsh, R. M. Susceptibility to cytotoxic T lymphocyte-induced apoptosis is a function of the proliferative status of the target. *J. Exp. Med.* **179:**769–774; 1994.
[143] Yang, J.-Q.; Li, S.; Domann, F. E.; Buettner, G. R.; Oberley, L. W. Superoxide generation in v-Ha-ras transformed human keratinocyte HaCaT cells. *Mol. Carcinog.* **26:**180–188; 1999.
[144] Sun, Y.; Oberley, L. W. Redox regulation of transcriptional activators. *Free Radic. Biol. Med.* **21:**335–348; 1996.
[145] Gonzalez-Flecha, B.; Demple, B. Biochemistry of redox signaling in the activation of oxidative stress genes. In: Gilbert, D. L.; Colton, C. A., eds. *Reactive oxygen species in biological systems*. New York: Kluwer Academic/Plenum Publishers; 1999: 133–154.
[146] Lee, Y. J.; Galoforo, S. S.; Berns, C. M.; Chen, J. C.; Davis, B. H.; Sim, J. E.; Corry, P. M.; Spitz, D. R. Glucose deprivation-induced cytotoxicity and alterations in mitogen-activated protein kinase activation are mediated by oxidative stress in multidrug-resistant human breast carcinoma cells. *J. Biol. Chem.* **273:**5294–5299; 1998.
[147] Blackburn, R. V.; Spitz, D. R.; Liu, X.; Galoforo, S. S.; Sim, J. E.; Ridnour, L. A.; Chen, J. C.; Davis, B. H.; Corry, P. M.; Lee, Y. J. Metabolic oxidative stress activates signal transduction and gene expression during glucose deprivation in human tumor cells. *Free Radic. Biol. Med.* **26:**419–430; 1999.
[148] Lee, Y. J.; Galoforo, S. S.; Sim, J. E.; Ridnour, L. A.; Choi, J.; Forman, H. J.; Corry, P. M.; Spitz, D. R. Dominant-negative Jun N-terminal protein kinase (JNK-1) inhibits metabolic oxidative stress during glucose deprivation in a human breast carcinoma cell line. *Free Radic. Biol. Med.* **28:**575–584; 2000.
[149] Daily, D.; Vlamis-Gardikas, A.; Offen, D.; Mittelman, L.; Melamed, E.; Holmgren, A.; Barzilai, A. Glutaredoxin protects cerebellar granule neurons from dopamine-induced apoptosis by activating NF-κB via Ref-1. *J. Biol. Chem.* **276:**1335–1344; 2001.
[150] Lewin, S. *Vitamin C: its molecular biology and medical potential*. London, New York: Academic Press; 1976.
[151] Rodkey, F. L.; Donovan, J. A. Oxidation-reduction potentials of the diphosphopyridine nucleotide system. *J. Biol. Chem.* **234:**677–680; 1959.
[152] Martin, R. B.; Edsall, J. T. Glutathione: ionization in basic solutions and molecular rearrangement in strongly acidic solutions. *Bull. Soc. Chim. Biol. (Paris)* **40:**1763–1771; 1958.
[153] Rabenstein, D. L. Nuclear magnetic resonance studies of the acid-base chemistry of amino acids and peptides. I. Microscopic ionization constants of glutathione and methylmercury-complexed glutathione. *J. Am. Chem. Soc.* **95:**2707–2803; 1973.
[154] Stenzel, J. D.; Welty, S. E.; Benzick, A. E.; Smith, E. O.; Smith, C. V.; Hansen, T. N. Hyperoxic lung injury in Fischer-344 and Sprague-Dawley rats in vivo. *Free Radic. Biol. Med.* **14:**531–539; 1993.
[155] Jones, D. P.; Carlson, J. L.; Samiec, P. S.; Sternberg, P.; Mody, V. C.; Reed, R. L.; Brown, L. A. S. Glutathione measurement in human plasma. Evaluation of sample collection, storage and derivatization conditions for analysis of dansyl derivatives by HPLC. *Clin. Chim. Acta* **275:**175–185; 1998.
[156] Kosower, N. S.; Kosower, E. M. The glutathione status of cells. *Int. Rev. Cytol.* **54:**109–161; 1978.
[157] Chatterjee, S.; Noack, H.; Possel, H.; Keilhoff, G.; Wolf, G. Glutathione levels in primary glial cultures: monochlorobimane provides evidence of cell type-specific distribution. *Glia* **27:**152–161; 1999.
[158] Wahllander, A.; Soboll, S.; Sies, H.; Linke, I.; Muller, M. Hepatic mitochondrial and cytosolic glutathione content and the subcellular distribution of GSH-S-transferases. *FEBS Lett.* **97:**138–140; 1979.
[159] Soboll, S.; Grundel, S.; Harris, J.; Kolb-Bachofen, V.; Ketterer, B.; Sies, H. The content of glutathione and glutathione S-transferases and the glutathione peroxidase activity in rat liver nuclei determined by a non-aqueous technique of cell fractionation. *Biochem. J.* **311:**889–894; 1995.
[160] Meister, A. Glutathione deficiency by inhibition of its synthesis and its reversal: applications in research and therapy. *Pharmacol. Ther.* **51:**155–194; 1991.
[161] Nishiuch, Y.; Sasaki, M.; Nakayasu, M.; Oikawa, A. Cytotoxicity of cysteine in culture media. *In Vitro* **12:**635–638; 1976.
[162] Anderson, M. E.; Meister, A. Intracellular delivery of cysteine. *Methods Enzymol.* **143:**313–325; 1987.
[163] Levy, E. J.; Anderson, M. E.; Meister, A. Transport of glutathione diethyl ester into human cells. *Proc. Natl. Acad. Sci. USA* **90:**9171–9175; 1993.
[164] Cotgreave, I. A. N-acetylcysteine: pharmacological considerations and experimental and clinical applications. *Adv. Pharmacol.* **38:**205–227; 1997.
[165] Williamson, J. M.; Meister, A. Stimulation of hepatic glutathione formation by administration of L-2-oxothiazolidine-4-carboxylate: a 5-oxo-L-prolinase substrate. *Proc. Natl. Acad. Sci. USA* **78:**936–939; 1981.
[166] Williamson, J. M.; Boettcher, B.; Meister, A. Intracellular cysteine delivery system that protects against toxicity by promoting glutathione synthesis. *Proc. Natl. Acad. Sci. USA* **79:**6246–6249; 1982.
[167] Vita, J. A.; Frei, B.; Holbrook, M.; Gokce, N.; Leaf, C.; Keaney, J. F. L-2-Oxothiazolidine-4-carboxylic acid reverses endothelial dysfunction in patients with coronary artery disease. *J. Clin. Invest.* **101:**1408–1414; 1998.
[168] Anderson, M. E.; Luo, J. L. Glutathione therapy: from prodrugs to genes. *Semin. Liver Dis.* **18:**415–424; 1998.
[169] Wuellner, U.; Seyfried, J.; Groscurth, P.; Beinroth, S.; Winter, S.; Gleichmann, M.; Heneka, M.; Loeschmann, P. A.; Schulz, J. B.; Weller, M.; Klockgether, T. Glutathione depletion and neuronal cell death: the role of reactive oxygen intermediates and mitochondrial function. *Brain Res.* **826:**53–62; 1999.

[170] Barbson, J. R.; Reed, D. J. Inactivation of glutathione reductase by 2-chloroethyl nitrosourea-derivatised isocyanates. *Biochem. Biophys. Res. Commun.* **83:**745–762; 1978.
[171] Woodward, G. E.; Hudson, M. T. The effect of 2-desoxy-D-glucose on glycolysis and respiration of tumor and normal tissues. *Cancer Res.* **14:**599–605; 1954.
[172] Laszlo, J.; Humphreys, S. R.; Goldin, A. Effects of glucose analogues (2-deoxy-D-glucose, 2-deoxy-D-galactose) on experimental tumors. *J. Natl. Cancer Inst.* **24:**267–280; 1960.
[173] Gordon, G.; Mackow, M. C.; Levy, H. R. On the mechanism of interaction of steroids with human glucose 6-phosphate dehydrogenase. *Arch. Biochem. Biophys.* **318:**25–29; 1995.
[174] Hollenberg, P. F. Mechanisms of cytochrome P450 and peroxidase-catalyzed xenobiotic metabolism. *FASEB J.* **6:**686–694; 1992.
[175] Sies, H.; Brigelius, R.; Wefers, H.; Muller, A.; Cadenas, E. Cellular redox changes and response to drugs and toxic agents. *Fundam. Appl. Toxicol.* **3:**200–208; 1983.
[176] Jones, D. P.; Maellaro, E.; Slater, A. F. G.; Orrenius, S. Effects of N-acetyl-L-cysteine on T-cell apoptosis are not mediated by increased cellular glutathione. *Immunol. Lett.* **45:**205–209; 1995.
[177] Cai, J. Y.; Wallace, D. C.; Zhivotovsky, B.; Jones, D. P. Separation of cytochrome c-dependent caspase activation from thiol-disulfide redox change in cells lacking mitochondrial DNA. *Free Radic. Biol. Med.* **29:**334–342; 2000.

ABBREVIATIONS

AT—3-amino-1,2,4-triazole
BCNU—Carmustine, 1,3-*bis*[2-chloroethyl]-1-nitrosourea
BSO—L-buthionine-SR-sulfoximine
CoQ—Coenzyme Q, ubiquinone
DHEA—Dehydroepiandrosterone
DTT—Dithiothreithol
EA—Ethacrynic acid
E, E°, E°′—Reduction potential at nonstandard conditions, standard condition (pH = 0), standard conditions (pH = 7)
E_{hc}—Half-cell reduction potential
GPx—Glutathione peroxidase
GR—Glutathione (disulfide) reductase
GS—Glutathione synthetase
GSH, GSSG—Glutathione, glutathione disulfide
NAC—N-acetyl-L-cysteine
NADH, NAD^+—Nicotinamide adenine dinucleotide, oxidized form
NADPH, $NADP^+$, $NADP^{\bullet}$—Nicotinamide adenine dinucleotide phosphate, oxidized form, radical
OTC—2-Oxothiazolidine-4-carboxylate
PSH/PSSP, PSSG—Proteinsufhydryl, protein disulfide
RNS—Reactive nitrogen species
ROS—Reactive oxygen species
SOD—Superoxide dismutase
$Trx(SH)_2$/TrxSS—Thioredoxin, thioredoxin disulfide