CHAPTER I

REDOX STATE AND REDOX ENVIRONMENT IN BIOLOGY

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1. INTRODUCTION

1.1 Cells have a reducing environment

Cells and tissues must maintain a reducing environment to survive. This reducing environment provides the electrochemical gradient needed for electron flow. This movement of electrons provides the energy needed to build and maintain cellular structures and associated functions. An array of redox couples is responsible for the electron transfer. Some of these redox couples are linked to each other to form sets 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 kinetically. 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 for the NAD^+/NADH and $NADP^+$/NADPH couples*.

It is now realized that the biological state of the cell changes with the reducing environment of the cell. [2] Many transcription factors are sensitive to redox changes in the cell. Thus, changes in the cellular redox environment can initiate signaling

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* **Abbreviations used:** BSO, L-Buthionine-SR-sulfoximine; CoQ, Coenzyme Q, ubiquinone; E, E^o, $E^{o'}$; … , 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; $Grx(SH)_2$/GrxSS, Glutaredoxin, glutaredoxin disulfide; 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; ROS, Reactive Oxygen Species; SOD, Superoxide dismutase; $Trx(SH)_2$/ TrxSS, Thioredoxin, thioredoxin disulfide.

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cascades. [3] The signaling by redox mechanisms relies on the chemistry that is driven by changes in the electrochemical potential of redox couples in the cell. Below we discuss how the electrochemical potentials of these redox couples are determined and indicate how these potentials influence cell signaling.

2. REDOX STATE AND REDOX ENVIRONMENT: A DEFINITION

Redox state is a term that has been used to describe the ratio of the interconvertible oxidized and reduced forms of a specific redox couple. For example, Sir Hans Krebs focused on the NAD^+/NADH couple and defined the redox state of this couple in a cell to be $[NAD^+]_{free}/[NADH]_{free}$. [4, 5, 6] 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.

> *The **redox state** of a redox couple is defined by the half-cell reduction potential and the reducing capacity of that couple.* [2]
>
> *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.*
>
> *Reducing capacity refers to the "size" of the pool (concentration) of reducing equivalents available, i.e. the strength of the redox buffer.* [2]

The above definitions attempt to clarify the difference between the redox state of a specific redox couple and the redox environment of a cell or tissue. When describing a specific redox couple the term redox state is appropriate. When describing changes in biological systems that involve various redox couples the term redox state should not be used, rather the term redox environment should be used.

3. THE NERNST EQUATION

The redox couples in cells and tissues can be viewed as electrochemical cells. 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 (Eqns. 1-3).

$$\Delta G^\circ = -n\,\mathbf{F}\,\Delta E^\circ , \qquad (1)$$

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, *i.e.*,

the difference in the standard reduction potentials of the two half-cells involved in the process. The superscript ° implies the thermodynamic standard state[†]. [7]

Using $\Delta G° = -n\mathbf{F}\Delta E°$, $\Delta G = -n\mathbf{F}\Delta E = \Delta G° + RT \ln Q$ and equation 6, the voltage of an electrochemical cell can be expressed as:

$$\triangle E = \triangle E^o - \frac{RT}{n\mathbf{F}} \ln Q \qquad \textbf{Nernst Equation} \qquad (2)$$

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:

$$\triangle E = \triangle E^o - \frac{59.1 \text{ mV}}{n} \log Q \qquad (3)$$

Thus, the Nernst equation can be used to determine the electrochemical potential between two redox couples. The electromotive force from these redox pairs (ΔE) is determined by subtracting the reduction potential of the species that is oxidized E_1 from the species that is reduced E_2:

$$\Delta E = E_2 - E_1 \qquad (4)$$

$$\begin{array}{lll} \text{Redox-couple 1} & Red_1 \longrightarrow & Ox_1 + e^- \\ \text{Redox-couple 2} & Ox_2 + e^- \longrightarrow & Red_2 \\ \hline \text{Redox-reaction} & Red_1 + Ox_2 \rightleftharpoons & Ox_1 + Red_2 \end{array} \qquad (5)$$

[†] 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 non-standard 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: $Ox + ne^- \rightarrow Red$ is a reduction potential; an oxidation potential corresponds to $Red \rightarrow Ox + ne^-$ as a half reaction. Here all values of E are reduction potentials unless otherwise noted.

$$\triangle E = \left(E^{o}_{2} - \frac{59.1\ mV}{n} \log \frac{[Red_2]}{[Ox_2]} \right) - \left(E^{o}_{1} - \frac{59.1\ mV}{n} \log \frac{[Red_1]}{[Ox_1]} \right) \quad (6)$$

If ΔE is zero, there is no net electron flow. When ΔE is not zero, the sign determines the direction of electron flow, *i.e.*, the direction of the redox reaction. The greater the magnitude of ΔE, the greater the thermodyndamic "pressure" pushing the reaction. Thus, as the redox state of couples such as GSSG/2GSH and $NADP^+$/NADPH change, they can force changes in other redox pairs, for example signaling proteins. These changes then can lead to biological consequences, such as proliferation, differentiation, apoptosis *etc*.

4. EXAMPLE REDOX REACTIONS

4.1 1e⁻-processes

Paraquat (PQ^{2+}) is a widely used pesticide, a nonselective contact herbicide, that has high pulmonary toxicity with no known antidote. Cellular enzyme systems readily reduce PQ^{2+} by one-electron reactions to $PQ^{\bullet+}$. This radical is very reducing; for the redox pair

$$PQ^{2+}/PQ^{\bullet+} \qquad E^{o\prime} = -448\ mV \quad pH\ 7.0. \quad (7)$$

$PQ^{\bullet+}$ has a strong tendency to donate its electron to other species, such as oxygen to produce superoxide,

$$PQ^{\bullet+} + O_2 \xrightarrow{k = 8 \times 10^8\ M^{-1}\ s^{-1}\ [8]} PQ^{2+} + O_2^{\bullet-} \quad (8)$$

There is a large thermodynamic driving force for this reaction as $E^{o\prime}_{O_2/O_2^{\bullet-}} = -160$ mV. [9, 10, 7] Thus, at standard conditions, $\Delta E^{o\prime} = (-160\ mV) - (-448\ mV) = +288$ mV. However, in the real world the concentrations of the species will not be 1 M; to make an estimate of the effective potential driving this reaction we use Eqn 6 with n = 1, *i.e.*;

$$\Delta E = (-160 - 59.1 \log [O_2^{\bullet-}]/[O_2]) - (-448 - 59.1 \log [PQ^{\bullet+}]/[PQ^{2+}])\ \text{in mV}. \quad (9)$$

If the steady-state level of superoxide in a cell is 10^{-10} M and $[O_2]$ is 10 μM (10^{-5} M), and the ratio of $[PQ^{\bullet+}]/[PQ^{2+}]$ is 1/10 000, then $\Delta E = +345$ mV. This positive potential is consistent with the observed rapid formation of superoxide by $PQ^{\bullet+}$.

The high flux of superoxide due to $PQ^{\bullet+}$ will lead to a high flux of hydrogen peroxide and consequently a large demand for reducing equivalents from GSH and NADPH, species that in general are two-electron donors. This will undoubtedly

result in a major change in the redox environment of cells and tissues exposed to paraquat, contributing to the toxicity of paraquat.

4.2 $2e^-$ -processes

Most redox reactions in biology are two-electron processes. Two-electron processes avoid formation of reactive intermediates such as free radicals. As with the $1e^-$ processes, there is a thermodynamic hierarchy for the two-electron redox reactions, Table 1. [11, 12, 13]

Table 1: Two-Electron Reduction Potentials

Redox Couple (2-electron reductions)	**E°'/mV at 25°C**	**Reference**
Xanthine/hypoxanthine, H^+	–371	13, 14
Uric acid/xanthine, H^+	–360	13
DTTox (cyclic disulfide), $2H^+$/DTT (Dithiotreitol)	–317[a]	15
NAD^+, H^+/NADH	–316	16, 13
$NADP^+$, H^+/NADPH	–315	16, 11
Lipoic acid, $2H^+$/Dihydrolipoic acid	–290	11, 17
GSSG, $2H^+$/2GSH (Glutathione)	–240	18, 19
Cys-S-S-Cys, $2H^+$/2Cys-SH (Cystiene)	–230	11, 20
TrxSS, $2H^+$/Trx(SH_2) (Thioredoxin)	-240[b]	18
GrxSS, $2H^+$/Grx(SH_2) (Glutaredoxin)	-218	18
FMN, $2H^+$/$FMNH_2$	–219	21
FAD, $2H^+$/$FADH_2$	–219	21
Riboflavin, $2H^+$/Leuco-riboflavin	–200	13, 12
Acetaldehyde, $2H^+$/ethanol	–197	13
Pyruvate, $2H^+$/lactate	–183	11
Oxaloacetate, $2H^+$/malate	–166	22
Methylene blue, $2H^+$/Leuco-methylene blue	+11	11, 12
Dehydroascorbate, H^+/Ascorbate	+54	23
Ubiquinone (CoQ),$2H^+$/ubihydroquinone ($CoQH_2$)	+84	24
O_2, $2H^+$/ H_2O_2	+ 300	7
NO_3^-/NO_2^-	+421	25
H_2O_2, $2H^+/2H_2O$	+1320	7

[a] This value assumes the reduction potential of NAD^+ is –316 mV at pH 7.0.
[b] This value can range from –270 to –124 mV. The value –240 mV is taken as typical for mammalian thioredoxin.

Some of the most important redox couples in biology are the glutathione system (GSSG/2GSH), the nicotinamide adenine dinucleotide phosphate system ($NADP^+$/NADPH) and the thioredoxin system ($TrxSS/Trx(SH)_2$). Glutathione is considered to be the major thiol-disulfide redox buffer of the cell. [26] NADPH is a major source of electrons for reductive biosynthesis and the source of electrons for the glutathione and thioredoxin systems. Thioredoxin is another important thiol-system in the cell. Among its many functions, thioredoxin reduces cystine moieties in the DNA-binding sites of several transcription factors and is therefore important in gene expression. [27, 28] All three redox couples transfer electrons through $2e^-$-processes, but the Nernst equation is different in each case. Note, the reduction potential for the two $1e^-$-steps cannot just be added because the value of E for a process depends not only on the number of electrons but also on the Gibbs energy changes for each step. For example:

a. $NAD^+ + e^- \rightarrow NAD^\bullet \quad E^{\circ\prime} = -913$ mV

b. $\underline{NAD^\bullet + e^- + H^+ \rightarrow NADH \quad E^{\circ\prime} = +282 \text{ mV}}$

c. $NAD^+ + 2e^- + H^+ \rightarrow NADH \quad E^{\circ\prime} = -316$ mV at 25°C, pH 7.0 (10)

For simplicity one can view the –316 mV as the average of the two one-electron reduction reactions. This value results because in reaction "c" two electrons are involved.

4.2.1 The $NADP^+$/NADPH system

The reduction half-reaction for the $NADP^+$, H^+/NADPH couple is:

$$NADP^+ + 2e^- + H^+ \longrightarrow NADPH$$

The Nernst equation for this process has the form:

$$E_{hc} = -315 - \frac{59.1}{2} \log \frac{[NADPH]}{[NADP^+]} \text{ mV at 25°C, pH 7.0} \ddagger \quad (11)$$

As an example, if we assume that in a cell NADPH is 80 μM and $NADP^+$ is 0.8 μM, then

$$E_{hc} = -315 - \frac{59.1}{2} \log \frac{[80 \times 10^{-6} \text{ M}]}{[0.8 \times 10^{-6} \text{ M}]} \text{ mV} \quad (12)$$

‡ In this chapter details on adjusting Nernst equation calculations for different pH values are not presented. A detailed discussion can be found in Ref [2].

Because the concentration units divide out, only the ratio of the concentrations of the two species in the redox pair are needed to determine E_{hc} of this couple. Thus, the actual units need not be molarity as is typically entered into the Nernst equation, but any form of concentration can be used as long as the units divide out.

In this example the ratio of NADPH/$NADP^+$ is 100:1, which is on the order of that found in various animal tissues. [29]

$$E_{hc} = -315 - \frac{59.1}{2} \log \frac{100}{1} = -374 \text{ mV} \quad (13)$$

The very negative reduction potential of –374 mV supports the idea that the $NADP^+$/NADPH couple is a major driving force for maintaining the reducing environment in cells and tissues.

4.2.2 The GSSG/2GSH system

In case of the GSSG, $2H^+$/2GSH couple knowing only the ratio [GSH]/[GSSG] does not allow the determination of the half-cell reduction potential for this couple. The reduction half-reaction for this couple is:

$$GSSG + 2H^+ + 2e^- \longrightarrow 2GSH \quad (14)$$

Note that **one** molecule of GSSG forms **two** molecules of GSH. Therefore, [GSH] will enter the Nernst equation as a squared term:

$$E_{hc} = -240 - \frac{59.1}{2} \log \frac{[GSH]^2}{[GSSG]} \text{ mV at 25°C, pH 7.0.} \quad (15)$$

As an example, if we assume that the concentration of GSH is 5 mM and that of GSSG is 50 μM, then the Nernst equation would be:

$$E_{hc} = -240 - \frac{59.1}{2} \log \frac{[5 \times 10^{-3} \text{ M}]\ [5 \times 10^{-3} \text{ M}]}{[5 \times 10^{-5} \text{ M}]} \quad (16)$$

Because [GSH] enters this equation as a squared term, the units will not divide out as in the case of NADPH. In this example if we enter the ratio of [GSH]/[GSSG] into the equation, the absolute molar concentration of GSH must still be known to completely specify the redox state of this couple:

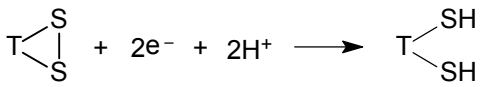
$$E_{hc} = -240 - \frac{59.1}{2} \log \frac{(100\ [GSH])}{1} \text{ mV} \quad (17)$$

Thus, in cases where reduction of the oxidized half of the couple results in the formation of two reduced molecules, as with GSH, the molar concentration needs to be known.

The GSSG/2GSH couple is thought of as the major redox buffer in the cell, while the more reducing $NADP^+$/NADPH couple with its lower concentration but more negative reduction potential provides a strong reducing force. Because GSH is present at mM levels in the cell, it can serve as a buffer for oxidative or reducing events, thereby keeping the cellular redox environment stable. The redox state of the GSSG/2GSH couple is now being considered as a possible reflection of the biological state of the cell. [30, 2] Signaling events probably contribute to the development of a particular redox state for this couple. Changes in the GSH pool might trigger signals for adaptive responses, such as proliferation, differentiation or death. Thus, the redox state of the GSSG/2GSH couple could both be a result of as well as a trigger for signaling cascades. Research in the next years will need to investigate the interdependence of the GSSG/2GSH couple and cell signaling events.

4.2.3 The Thioredoxin, TrxSS/Trx(SH)$_2$, system

The thioredoxins are a family of low molecular weight (≈11-12 kDa) dicysteine proteins that in contrast to the GSH system use a macromolecular structure to transfer electrons. Their redox chemistry is characterized by two intra-molecular sulfhydryl groups that can be oxidized to form internal disulfide bonds. The reduction half-reaction for this redox pair is:



(18)

One molecule TrxSS forms **one** molecule $Trx(SH)_2$ and the Nernst equation for the thioredoxin half-cell potential has the same form as that of the $NADP^+$/NADPH couple.

$$E_{hc} = E^{\circ\prime} - \frac{59.1}{2} \log \frac{[T(SH)_2]}{[TSS]} \qquad \text{mV at 25°C, pH 7.0} \qquad (19)$$

The thioredoxins are more specific than the glutathione system. They are known to react with approximately 20-30 biomolecules that are involved in regulatory and catalytic processes. [31] Thioredoxin modulates the activity of transcription factors such as Ref-1 (that regulates AP-1), NF-κB and the glucocorticoid receptor. [32] It also plays a role in the regulation of stress kinases, such as ASK1, thereby regulating stress signaling cascades.

4.2.4 When push comes to shove

The two electron processes discussed above are an example of linked cellular redox couples. Both the Trx and GSH-systems use NADPH as a source of reducing equivalents; thus, they are thermodynamically connected to each other. **Figure 1** clearly shows why NADPH is the thermodynamic driving force for both the thioredoxin and GSH systems. Because of the much higher concentration of GSH compared to NADPH and $Trx(SH)_2$, it has been the focus for research on how the redox environment of cells is connected to the biological state of the cell and cell signaling processes. As seen in the Nernst equation, simply reporting the total GSH or the ratio GSH/GSSG does not convey complete information on the redox state of this couple. One must specify both E_{hc} for this couple and [GSH].

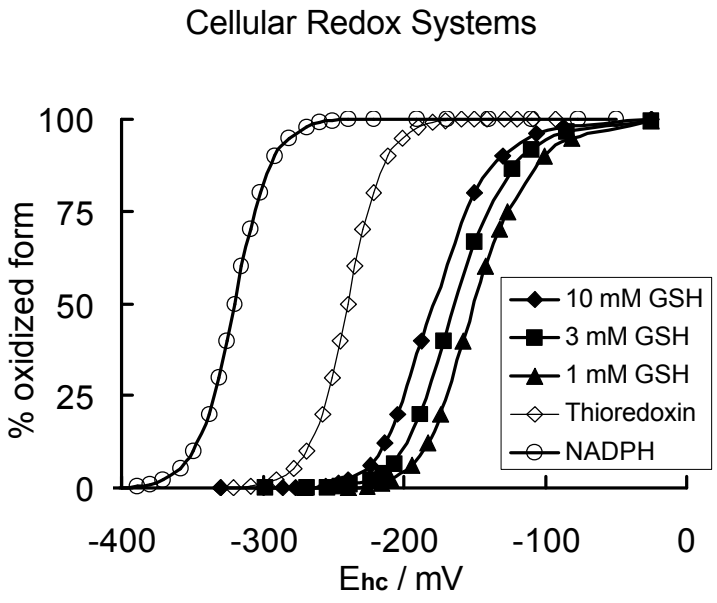


Figure 1. The $NADP^+$/NADPH couple provides the reducing equivalents needed for the thioredoxin and GSH system. The thioredoxin E°' is taken as –240 mV. For the $NADP^+$/NADPH couple to be an efficient source of reducing equivalents for the GSH system these two systems should not be at thermodynamic 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. [2] Thus, these two redox pairs, which are connected by glutathione disulfide reductase, appear to be out of equilibrium by a factor of 1000 or more. This is to be expected. If they were in equilibrium, then there would be no driving force to maintain an appropriate pool of GSH with a minimum amount of GSSG.

To convey all this information in a simple way we suggest use of the following notation for the status of a redox pair, such as GSSG/2GSH,

$$\{E_{hc}(GSH);\ [GSH]\},\ \textit{e.g.}\ \{-187\ mV\ (GSH);\ 3.5\ mM\}.$$

Here −187 mV is the actual half-cell reduction potential of the GSSG/2GSH couple in the setting of interest and 3.5 mM is the concentration of GSH, the reduced species in the couple. With this information all elements of this redox pair are known.

6. PRACTICAL APPROACHES FOR DETERMINING REDOX ENVIRONMENT

As seen above, the half-cell potential of redox couples is pH-dependent, temperature-dependent, and sometimes requires molar concentrations rather than concentration ratios. Thus, to determine the E_{hc} of the GSSG/2GSH couple in cells the intra cellular pH, the temperature and the volume of the cell needs to be assessed. Intracellular pH can be measured using fluorescence probes with a flow cytometer. [33, 34, 35, 36, 37] The volume of the cell can be determined using a Coulter Counter. Once the volume is known, the concentration of the species in the redox couple can be determined.

If these techniques are not available, a simpler approach using the resting state of a cell as reference, can be used to estimate changes in redox potentials. An example for the GSSG/2GSH couple is given by Antunes *et al.* [38] In this approach the GSH and GSSG levels are measured in the "resting" state of the cell and then upon treatment. These values can then be entered into the Nernst equation as formulated in Eqn. 20.

$$\Delta E_{hc} = E_{hc(treatment)} - E_{hc(resting\ state)}$$

$$\Delta E_{hc} = \{E^{o\prime} - (RT/n\mathbf{F})\ \ln\ ([GSH]^2/[GSSG])_{(treatment)}\} - \{E^{o\prime} - (RT/n\mathbf{F})\ \ln\ ([GSH]^2/[GSSG])_{(resting\ state)}\}$$

$$\Delta E_{hc} = \frac{RT}{n\mathbf{F}}\ \ln\ \{(GSSG]/[GSH]^2)_{(treatment)} \bullet ([GSH]^2/[GSSG])_{(resting\ state)}\} \qquad (20)$$

Using this method, all the concentration units for the glutathione couple divide out. Thus, any means of expressing concentration can be used as long as those for GSSG in the two states are the same units and the units for the GSH levels in the two states are the same. This approach will not yield a half-cell potential for the redox couple, but rather it provides a measure of the change in the redox state of this couple, which should reflect the change in the overall cellular redox environment. Please note that in this approach two principal assumptions are made:

1. The volume of the cells does not change with treatment, and
2. The intracellular pH is the same in the resting and treatment states.

This approach can provide a great deal of quantitative information when studying the toxicological effects of substances on cells.

7. REDOX ENVIRONMENT, CONSIDERATIONS AND MEASUREMENTS

7.1 Redox Environment and the Redox State of the GSH Couple

It is now being recognized that the redox state of the glutathione system may be involved in the determination of the biological state of the cell. [2, 30, 39] In general it appears that high levels of GSH and the usually associated low E_{hc} are affiliated with proliferation; increases in E_{hc} will slow proliferation and increase differentiation; additional increases in E_{hc} can suppress differentiation and bring about apoptosis or if severe, necrosis.

Several examples in the literature now exist that demonstrate quantitatively that an increase in E_{hc} can result in apoptosis. Antunes *et al.* found that initiation of apoptosis through H_2O_2 was accompanied by a shift in the redox environment towards more oxidizing E_{hc}. [38] Cai *et al.* have demonstrated that toxicants that increase E_{hc} of the GSSG/2GSH couple by ≈70 mV from control cells can initiate apoptosis. [40]

Findings in the GSH-redox field often seem confusing and contradicting. One treatment might change the biological status of one cell line or tissue while bringing about no response in another cell line. One of the reasons might be that the concentration of GSH varies from cell line to cell line. As seen in Figure 1, to make a substantial change in the redox environment of an organism that has 10 mM GSH *vs.* 1 mM GSH requires a much greater oxidative assault. Thus, when examining the influence of redox stress on biological events one should take the total concentration, or more importantly, the redox-buffer capacity into account.

7.2 Redox Environment and Signaling

A signal is like a speck of methylene blue entering water. If the small speck of dye is put into a small puddle of water, an intense color is observed, but if it is put into the ocean the initial small bit of evidence that methylene blue is there is soon gone as it is diluted; no color change will be visible. The GSH buffer can be viewed similarly. If a signal enters a cellular GSH buffer with low capacity it will be "visible" and result in activation of transcription factors or initiation of signaling cascades. If the GSH buffer capacity is very high, the signals might be dampened and never reach their endpoint. Thus, the GSH buffer could influence the strength and duration of a signal, but might not determine which individual signaling process occurs. [41] The concept is similar when investigating overall changes in biological status, such as a shift from proliferation to cell death. If the redox state of the

GSSG/2GSH couple is close to the border where apoptosis can occur, then a weak signal (small change in GSH concentration) can initiate this shift. Thus, it is important to consider the capacity of the cellular redox-buffer.

Another concern is that all processes are considered and appropriately examined. An example is provided by Hansen *et al*. in their studies on the modulation of differentiation by changes in the GSH. [42] Treatment of rabbit limb bud cultures with BSO lead to apparent inhibition of differentiation, while treatment with N-acetylcysteine (NAC) appears to restore differentiation, an observation that seemed inconsistent with the generalization on GSH levels outlined in section 7.1 above. However, when these investigators considered each stage of this process, their observations were consistent with the generalizations. Their experience points to a need for careful examination of processes before conclusions can be appropriately made.

Hansen *et al*. also compared rat tissue with rabbit tissue and found contrasting results. Because rat tissue had 43% more GSH than the rabbit tissue, the differing results could be due to a difference in GSH buffer capacity as mentioned above.

Many researchers consider the initiation of signaling processes to be a local event that require local gradients in the electrochemical potential of the redox pairs involved in the signaling process. Absolute quantitation of local cellular gradients of the redox state of specific redox couples is not yet possible. Some beginning approaches to qualitative imaging are underway. A most interesting study is the use of electron paramagnetic imaging of redox gradients in tumors. [43] Using a mouse model and the rate of nitroxide destruction, this study was able to provide spatially resolved, quantitative data on the redox environment of normal and tumor tissue and the influence of GSH on tissue redox status. This study clearly demonstrates that tumors have significant heterogeneity in their redox environment compared with normal tissue. In addition the RIF-1 tumor model used had about 4-fold greater GSH than normal tissue and this was reflected in the image. These initial studies point to more general approaches that may developed to examine quantitatively redox status and redox gradients in cells and tissues.

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