ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 7 & 8, 2006

Forum Review

Plasma Membrane Oxidoreductases: Effects on Erythrocyte Metabolism and Redox Homeostasis

ELEANOR C. KENNETT and PHILIP W. KUCHEL

ABSTRACT

Plasma membrane oxidoreductases (PMORs) have been found in the membranes of all cells. These systems have been studied extensively in the human erythrocyte, so much is known about their activity and effect on erythrocyte cellular functioning. PMORs have been shown to be involved in a number of events associated with cell growth and function in other cell lines, but perhaps their most important role, especially in the nucleus-free mature erythrocyte, is as a redox sensor. The PMOR reduces extracellular oxidants by using the reducing power of intracellular antioxidants, making the cell metabolism respond to changes in the local redox environment. Thus, the activity of the PMOR is closely linked to the metabolic status of the erythrocyte. The main intracellular reductant for this system is ascorbic acid; however, the cell must also have the ability to supply NADH for full activity. Nuclear magnetic resonance studies on the effects of extracellular oxidants on intracellular metabolism have increased our knowledge of the intimate link between PMOR activity and metabolism, and these studies are reviewed here in detail. *Antioxid. Redox Signal.* 8, 1241–1247.

INTRODUCTION

THE HUMAN ERYTHROCYTE is constantly exposed to sources of reactive oxygen species through its role as the oxygen and carbon dioxide transporter in the body. The composition of the erythrocyte plasma membrane and the surrounding milieu means that these cells are susceptible to oxidative damage (9). The presence of transmembrane electron transport systems in the erythrocyte plasma membrane provides the cell with an extra level of defense against extracellular oxidants. Plasma membrane oxidoreductases (PMORs) have been identified in all cell types investigated and have been purported to play a role in cell growth and proliferation, hormone responses, and other cell-signalling events (1); but perhaps their most important role, especially in erythrocytes, is to enable the cell respond to changes in both intra- and extracellular redox environments. The human erythrocyte plasma membrane contains at least one such electron-transport system, and it transfers reducing equivalents from intracellular antioxidants to extracellular oxidants (17). Erythrocyte PMORs have been investigated by using a number of different extracellular oxidants (see later), and several different reduction activities occur in the membrane. The common feature of all these PMORs, however, is the use of intracellular reductants, and as such, the activity of these systems is closely linked to the metabolic status of the erythrocyte. This review seeks to unite our current knowledge about how PMOR activity and metabolic activity in the erythrocyte are interrelated, with a special emphasis on the use of nuclear magnetic resonance (NMR) spectroscopy to elucidate metabolic effects in conditions that are close to *in vivo*.

ERYTHROCYTE PMOR ACTIVITY

Traditionally PMOR activities were named after the oxidant used to identify them and the purported intracellular electron donor (*e.g.*, NADH:ferricyanide oxidoreductase). This has led to some confusion over how many different PMORs exist. As the majority of PMOR activities have been identified by using nonphysiologic electron acceptors, it is highly likely that each PMOR is able to reduce a number of

School of Molecular and Microbial Biosciences, University of Sydney, NSW 2006, Australia.

different oxidants albeit with different affinities (17). In this review, PMOR activities are designated on the basis of the extracellular oxidant used (*e.g.*, ferricyanide reductase), and the possible intracellular donors are discussed in this context.

Ferricyanide reductase

The predominant electron acceptor used to investigate erythrocyte PMORs is the membrane-impermeable oxidant ferricyanide. Erythrocyte PMOR activity was initially discovered by using ferricyanide as the oxidant (11), and it has been widely used since. The reduction of ferricyanide $[Fe(CN)_6]^{3-}$ to ferrocyanide $[Fe(CN)_6]^{4-}$ occurs at the cell membrane and can be readily monitored spectrophotometrically (ϵ_{420nm}= 1 m*M*/cm); however, to obtain information on the metabolic effects of its reduction, multiple experiments must be performed. The application of NMR to the investigation of ferricyanide reduction with the ^{13}C-labeled form, $[Fe(^{13}CN)_6]^{3-}$, enabled the simultaneous measurement of ferricyanide reduction and changes to intracellular metabolism (14).

The reduction of extracellular ferricyanide by cells was originally attributed to the transfer of reducing equivalents from the intracellular redox compound NADH (48); however, NMR experiments in our laboratory (14) and investigations into the role of ascorbate in PMOR activity, predominantly performed by May *et al.* (22–29), indicate that NADH is not the sole or even the main source of reducing equivalents for ferricyanide reduction under usual conditions. The main source of reducing equivalents is thought to be ascorbate (29). Ascorbate and its two-electron oxidized form dehydroascorbate (DHA) are both able to stimulate the reduction of extracellular ferricyanide (14, 46), predominantly through increasing the intracellular ascorbate concentration (13). The role of ascorbate in PMOR activity has been reviewed in detail previously (22), so it is not discussed further here.

Ascorbate free radical (AFR) reductase

Transmembrane AFR reductase activity has also been identified in the erythrocyte plasma membrane (24, 25, 44, 45). This activity, which brings about the recycling of extracellular ascorbate from its oxidized derivative AFR, may represent a physiologic function of PMORs (44). The reduction of AFR is an electrogenic process, causing a depolarization of the membrane (44), paralleling that previously observed with ferricyanide (45). However, AFR reduction, unlike ferricyanide reduction, is insensitive to *p*-chloromercuribenzene sulfonate (pCMBS) (24, 25). The electrogenic property of the PMOR suggests that the mechanism of electron transport does not solely rely on the movement of small electron carrier molecules through the membrane, such as coenzyme Q, and it has been suggested that AFR reduction involves membrane cytochromes (44). However, it has been shown that removal of coenzyme Q from liver plasma membranes decreases the extent of AFR reduction (47). The authors suggest that this implies the involvement of both coenzyme Q and its reductase in the mechanism of transmembrane electron transport. Although the main source of reducing potential for AFR reduction comes from ascorbate (44), the reduction rate is intimately linked to the NADH content of the cell. NADH-mediated reduction of AFR displays both transmembrane and exofacial activities, with ~12% of the activity being transmembranous (25). The ascorbate-mediated reduction of extracellular AFR most likely depends on ascorbate-recycling mechanisms (Fig. 1), with a dependency on reduced glutathione (24, 26).

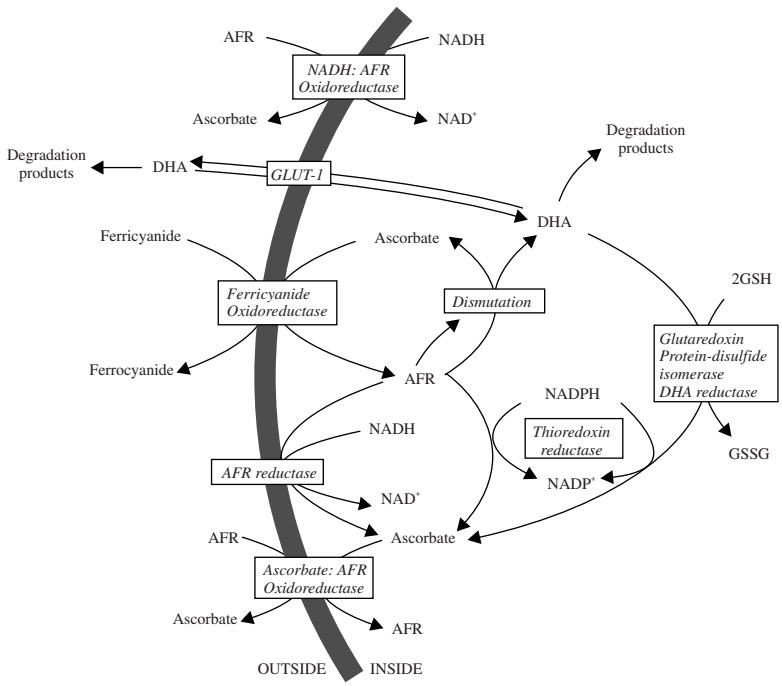

FIG. 1. Erythrocyte ascorbate recycling. *Arrowheads* show the main direction of electron flow under usual operation. The flux of DHA across the membrane through GLUT-1 is fast in both directions, allowing the rapid exchange of redox equivalents across the membrane. Based on figures in References 26 and 44.

Water-soluble tetrazolium-1 (WST-1) reductase

WST-1 is a membrane-impermeable tetrazolium redox dye that has been used extensively by Berridge *et al.* (2–4, 40) to investigate PMOR activity in a number of transformed and nontransformed cell lines. It is reduced at the plasma membrane, and two distinct PMOR activities have been identified: a cell-surface NADH oxidase activity that reduces WST-1 in the presence of NADH (3), and a transmembrane NADH oxidase activity that reduces WST-1 in the presence of phenazine methosulfate (PMS) (2,4). Both activities are inhibited by superoxide dismutase, indicating the involvement, direct or otherwise, as an electron donor (reductant) (3).

It was previously reported that erythrocyte membranes do not contain a WST-1 reductase activity (2). However, we have identified such a WST-1 reductase activity in human and other mammalian erythrocyte plasma membranes (Fig. 2). This transmembrane activity (identified in the presence of PMS) is inhibited by iodoacetate (61%) and superoxide dismutase (74%) (15). The human erythrocyte WST-1 reduction rate of 4.53 ± 0.6 mA_{450}/min (4×10^9 cells)/ml is significantly lower than activities previously reported from other cell lines, which lie in the range of 0.4 ± 0.2 – 45.6 ± 5.8 mA_{450}/min (3×10^5 cells)/ml (4). Despite the lower levels of activity, erythrocyte WST-1 reduction shows sensitivities to inhibitors similar to those reported in other cell lines (3–5).

^{1}H-NMR studies of WST-1 reduction indicated that during its reduction, WST-1 is reversibly bound to the plasma membrane with a mean residence time that is similar to its longitudinal relaxation time [*i.e.*, the NMR timescale (15)]. Thus, unlike ferricyanide (14), the reduction of WST-1 cannot be monitored by using NMR; but changes in intracellular metabolism are measurable by this means, and the results are discussed in the following sections.

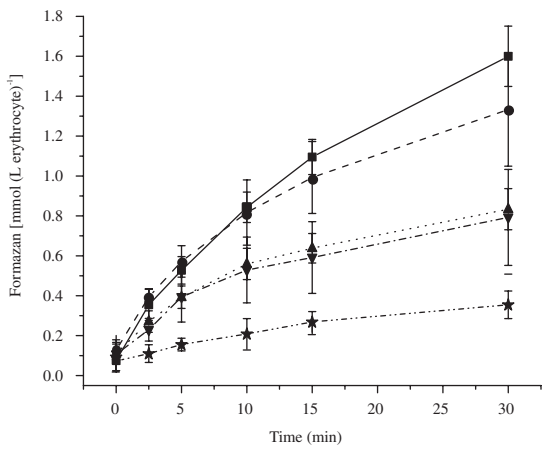

FIG. 2. WST-1 reductase activity by erythrocytes from different mammalian species. Erythrocytes (40% hematocrit) from cattle (▲, n = 6), horse (▼, n = 7), human (■, n = 6), kangaroo (*, n = 4), and sheep (●, n = 8) were incubated in 2 m*M* phosphate-buffered saline, pH 7.4, containing 5 m*M* glucose, 5 m*M* WST-1, and 5 μ*M* PMS. Data points are the average of *n* individuals within each species. Error bars denote ±1 standard deviation. Reproduced from Reference 15.

METABOLIC LINKS TO PMOR ACTIVITY

Glycolytic activity

Recent research into the characterization of the location of glycolytic enzymes has indicated that these enzymes may be organized into multimeric complexes near the amino terminus of band 3 (6). The assembly appears to be regulated by oxygenation and phosphorylation of band 3, with a closer association occurring in deoxygenated erythrocytes. This association of erythrocyte glycolytic enzymes with the plasma membrane suggests that an intimate link may exist between PMOR activity and metabolic control. Some evidence indicates that ATP made through the glycolytic pathway is channeled to membrane ion-pumps without it diffusing more widely throughout the cytoplasm (6). A similar scenario could be envisaged for the transfer of reducing equivalents across the membrane.

The application of NMR in studies of ferricyanide reduction provided many insights into the link between membrane redox activity and metabolic control (14). Himmelreich and Kuchel (14), by using a combination of ^{13}C-labeled compounds and ^{1}H-, ^{13}C-, and ^{31}P-NMR, investigated the effects of ferricyanide reduction on glycolytic activity, in real time. They observed that glucose was essential for ferricyanide reduction; however, the reduction of ferricyanide did not lead to a significant increase in the rate of glucose consumption above controls, 1.22 ± 0.05 compared with 1.20 ± 0.05 mmol/(L erythrocytes)/h, or in a substantial change to the lactate-to-pyruvate ratio (14). This was in agreement with the previous observation that intracellular NADH concentrations are relatively unaffected by erythrocyte exposure to ferricyanide (30). The presence of ferricyanide did, however, cause small changes in the flux through the Embden–Meyerhof pathway (decrease of 4%), pentose phosphate pathway (increase of 4%), and Rapoport–Luebering shunt (decrease of 5%). This change in the metabolic flux in the face of oxidative stress implies that more ATP will be produced per glucose molecule than is observed in controls (14). This outcome has also been seen in other studies (19, 33), possibly indicating that ATP is involved in the regulation of PMOR activity. No other studies have provided such detailed information about the effect of ferricyanide reduction on glycolytic flux. Inhibition of glycolytic enzymes has also been shown to decrease the rate of ferricyanide reduction (14, 21, 33, 39).

The extracellular reduction of WST-1 by erythrocytes causes a transient decrease in the output of lactate, as gauged from the lactate-to-pyruvate ratio (Fig. 3). This comes about from a relative increase in pyruvate production, suggesting that WST-1 reduction diverts NADH reducing equivalents from the glycolytic pathway. The effect of ascorbate on WST-1 reduction by erythrocytes was not tested; however, it would be interesting to compare it with the ferricyanide reductase activity. In the course of WST-1 reduction, the reduced-to-oxidized glutathione ratio decreases compared with controls (15). This oxidation of glutathione suggests that either NADPH is a rate-limiting factor, possibly through the action of PMS, which can produce intracellular superoxide in erythrocytes (20), or that flux through the oxidative pentose phosphate pathway (oPPP) is reduced by glycolysis, responding to an increased requirement for NADH.

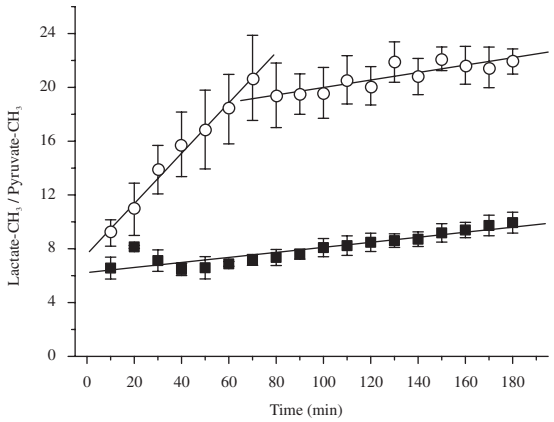

FIG. 3. Extracellular WST-1 effect on the metabolic rate of human erythrocytes. Erythrocytes (65% hematocrit) in 2 m*M* phosphate-buffered saline, pH 7.4, containing 5 m*M* glucose, were incubated at 37°C in the absence (○) and presence (■) of 5 m*M* WST-1 and 5 μ*M* PMS, and ^{1}H-NMR spectra were recorded. Data points represent the average relative integral of the lactate and pyruvate CH_3 peaks from three separate experiments ±1 standard deviation, and they reflect the relative concentrations of the two metabolites. Reproduced from Reference 15.

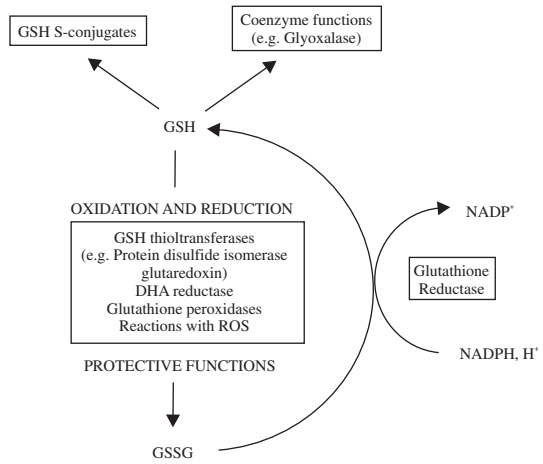

FIG. 4. Functions of glutathione within the erythrocyte.

Glutathione redox balance

Under conditions of oxidative stress, flux through the oPPP increases (41). This increase, once thought to be controlled by glucose-6-phosphate dehydrogenase activity, is actually controlled by hexokinase when NADPH concentrations are very low (41). Thus, changes in the intracellular glutathione redox status reflect flux through the oPPP. Glutathione can exist in three states, reduced (GSH), oxidized (GSSG), and in glutathione mixed disulfides (GSSR) with proteins or other thiol-containing molecules. Under normal conditions, >95% of the total erythrocyte glutathione is reduced (42), and it is maintained in this state by the NADPH-dependent enzyme glutathione reductase. A change in the glutathione redox status has repercussions on the functioning of the cell, and as such, its redox status has often been used as a "litmus test" for oxidative stress (Fig. 4). This is mainly due to its role as a cofactor for the detoxifying enzymes glutathione peroxidase, glutathione-*S*-transferase, and glyoxalase I (10, 37), its protection against reactive oxygen species (31), and its enhancement of PMOR activity in the face of an oxidative assault (27).

The responsiveness of glutathione to extracellular and intracellular oxidants has suggested to a number of researchers that it may be able to act extracellularly to protect the cell from an oxidative insult (7, 8, 12, 16, 32, 35, 36). In erythrocytes, whether extracellular GSH could influence the intracellular glutathione redox balance was not clear until recently. A combination of ^{1}H- and ^{13}C-NMR experiments used the glutathione isotopomer [*glycyl*-2-^{13}C]glutathione. This compound enabled intra- and extracellular, reduced and oxidized glutathione to be distinguished in a single series of experiments that showed that extracellular GSH has no effect on either the intracellular glutathione redox status or the metabolic rate (16). Previous studies have not used methods that allowed direct separation of the spectral peaks from the intra- and extracellular glutathione (7, 38). Their quantification of the two populations had previously been based on the error-prone procedure of subtracting one spectrum from another.

NAD(H) redox balance

The PMOR has been shown to require the availability of NADH for activity, as discussed earlier, and promotes flux through the glycolytic pathway, as evidenced by an increased output of ATP (19, 33). The NAD-to-NADH ratio has been shown to be important in the regulation of glycolysis (43); the forward flux through glyceraldehyde-3-phosphate dehydrogenase requires a high NAD-to-NADH ratio, and if it decreases, this clearly biases the equilibrium toward the upstream reactants (43). Maintenance of the normal NAD-to-NADH ratio (and consequently the lactate-to-pyruvate ratio) is one of the main regulators of glycolysis and not the absolute concentration of any of these molecules.

The effect of decreasing the NAD-to-NADH ratio (by increasing the intracellular NADH concentration) on the ability of the erythrocyte to respond to oxidative stress has been investigated in a number of *in vitro* studies (18, 34, 43, 49). Increasing the intracellular NADH concentration by exposure of erythrocytes to extracellular NADH leads to an increase in the reduction of nitrite-induced methemoglobin (18, 49). As a counterpart, decreasing the intracellular NADH concentration diminishes the ability of the cell to reduce methemoglobin (49). The use of extracellular NADH to increase intracellular pools depends on the availability of lactate dehydrogenase (LDH) activity. Isolated erythrocytes from species that have low endogenous LDH activity are unable to enhance their NADH levels without the addition of exogenous, extracellular LDH (18). The reaction scheme for this situation is given in Fig. 5. The extent to which this reaction scheme, observed *in vitro*, occurs *in vivo* is unknown.

The sensitivity of the NADH-cytochrome b_5 methaemoglobin reductase activity to the NAD-to-NADH ratio—higher

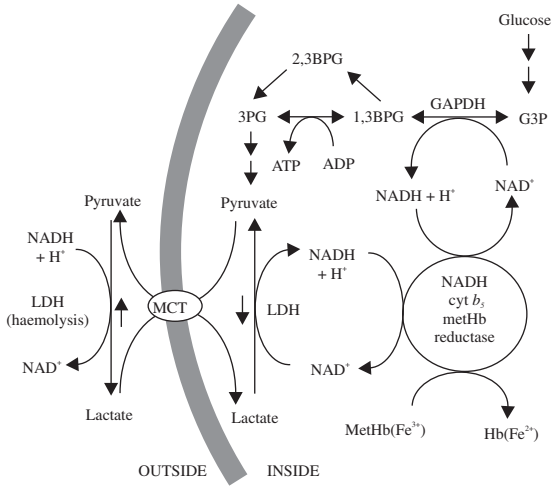

FIG. 5. Reaction scheme for the reduction of methemoglobin by extracellular NADH. 1,3BPG, 1,3-bisphosphoglycerate; 2,3BPG, 2,3-bisphosphoglycerate; G3P, glyceraldehyde 3-phosphate; Hb, hemoglobin; MCT, monocarboxylate transporter; and 3PG, 3-phosphoglycerate. Adapted from Fig. 4 in Reference 18.

activity when increased availability of NADH is present (18)—suggests that the PMOR activity, which also shows a dependence on the availability of NADH, may also be modulated by increasing the intracellular NADH pool size. This knowledge may be useful in determining when these systems are active under *in vivo* conditions and hence suggest targets for enhancing or inhibiting PMOR activity.

CONCLUDING REMARKS

The intimate link between PMOR activity and metabolic activity is well established. The challenge now is to identify the physiologic oxidants that are reduced by this system, under what circumstances the PMOR is activated, what controls exist over its activation, and how its activation changes cellular and system metabolism in an *in vivo* setting. The current knowledge of PMOR activity in the erythrocyte paves the way for these future areas of research on other cell types. Establishing the nature of the PMOR (*i.e.*, its composition; protein, small molecular, or a combination of both) will answer some of these questions, and undoubtedly the interaction that PMOR activity has with other metabolic reactions and *vice versa* will be more firmly established.

ACKNOWLEDGMENTS

This work was supported by grants from the Australian Research Council and the National Health and Medical Research Council to P.W.K. E.C.K received an Australian Commonwealth Postgraduate Award. We thank Drs. B. E. Chapman and W. A. Bubb for input in relation to NMR, and Mr. W. G. Lowe for expert technical assistance.

ABBREVIATIONS

AFR, ascorbate free radical; DHA, dehydroascorbate; GSH, reduced glutathione; GSSG, oxidized glutathione; GSSR, glutathione mixed disulfide; LDH, lactate dehydrogenase; oPPP, oxidative pentose phosphate pathway; PMOR, plasma membrane oxidoreductases; PMS, phenazine methosulfate; NMR, nuclear magnetic resonance; WST-1, water-soluble tetrazolium-1.

REFERENCES

1. Baker MA and Lawen A. The function of the plasma membrane NADH-oxidoreductase system: a critical review of the structural and functional data. *Antioxid Redox Signal* 2: 197–212, 2000.
2. Berridge MV and Tan AS. Trans-plasma membrane electron transport: a cellular assay for NADH- and NADPH-oxidase based on extracellular, superoxide-mediated reduction of the sulfonated tetrazolium salt WST-1. *Protoplasma* 205: 74–82, 1998.
3. Berridge MV and Tan AS. Cell surface NAD(P)H-oxidase: relationship to transplasma membrane NADH-oxidoreductase and a potential source of circulating NADH-oxidase. *Antioxid Redox Signal* 2: 277–288, 2000.
4. Berridge MV and Tan AS. High capacity redox control at the plasma membrane of mammalian cells: trans membrane, cell-surface and serum NADH-oxidases. *Antioxid Redox Signal* 2: 231–242, 2000.
5. Berridge MV, Tan AS, McCoy KD, and Wang R. The biochemical and cellular basis of cell proliferation assays that use tetrazolium salts. *Biochemica* 4: 15–20, 1996.
6. Campanella ME, Chu H, and Low PS. Assembly and regulation of a glycolytic enzyme complex on the human erythrocyte membrane. *Proc Natl Acad Sci U S A* 102: 2402–2407, 2005.
7. Ciriolo AR, Paci M, Sette M, de Martino A, Bozzi A, and Rotilio G. Transduction of reducing power across the plasma membrane by reduced glutathione. *Eur J Biochem* 215: 711–718, 1993.
8. Ciriolo MR, Sette M, Paci M, and Rotilio G. A study of the intracellular effects of glutathione by H-1-spin echo NMR of intact human erythrocytes. *Biochem Int* 20: 397–403, 1990.
9. Clemens MR and Waller HD. Lipid peroxidation in erythrocytes. *Chem Phys Lipids* 45: 251–268, 1987.
10. Cotgreave IA and Gerdes RG. Recent trends in glutathione biochemistry—glutathione-protein interactions: a molecular link between oxidative stress and cell proliferation? *Biochem Biophys Res Commun* 242: 1–9, 1998.
11. Dormandy TL and Zarday Z. The mechanism of insulin action: the immediate electrochemical effects of insulin on red-cell systems. *J Physiol (Lond)* 180: 684–707, 1965.
12. Guarnieri C, Fraticelli A, Ventura C, Vaona I, and Budini R. External GSSG enhances intracellular glutathione in isolated cardiac myocytes. *Biochem Biophys Res Commun* 147: 658–665, 1987.
13. Himmelreich U, Drew K, Serianni AS, and Kuchel PW. ^{13}C NMR studies of vitamin C transport and its redox cycling

in human erythrocytes. *Biochemistry* 37: 7578–7588, 1998.
14. Himmelreich U and Kuchel PW. ^{13}C-NMR studies of transmembrane electron transfer to extracellular ferricyanide in human erythrocytes. *Eur J Biochem* 246: 638–645, 1997.
15. Kennett EC. Transmembrane electron transport systems in erythrocyte plasma membranes. PhD thesis, School of Molecular and Microbial Biosciences, University of Sydney, 2004, pp 218.
16. Kennett EC, Bubb WA, Bansal P, Alewood P, and Kuchel PW. NMR studies of exchange between intra- and extracellular glutathione in human erythrocytes. *Redox Rep* 10: 83–90, 2005.
17. Kennett EC and Kuchel PW. Redox reactions and electron transfer across the red cell membrane. *IUBMB Life* 55: 375–385, 2003.
18. Kennett EC, Ogawa E, Agar NS, Godwin IR, Bubb WA, and Kuchel PW. Investigation of methaemoglobin reduction by extracellular NADH in mammalian erythrocytes. *Int J Biochem Cell Biol* 37: 1438–1445, 2005.
19. Maneva A, Taleva B, and Maneva L. Lactoferrin-protector against oxidative stress and regulator of glycolysis in human erythrocytes. *Z Naturforsch [C]* 58: 256–262, 2003.
20. Maridonneau I, Braquet P, and Garay RP. Na^+ and K^+ transport damage induced by oxygen free radicals in human red cell membranes. *J Biol Chem* 258: 3107–3113, 1983.
21. Marques F, Crespo ME, and Bicho M. Control of NADH ferricyanide reductase activity in the human erythrocyte by somatotrophin and insulin. *Redox Rep* 1: 113–117, 1995.
22. May JM. Is ascorbic acid an antioxidant for the plasma membrane? *FASEB J* 13: 995–1006, 1999.
23. May JM and Qu ZC. Ascorbate-dependent electron transfer across the human erythrocyte membrane. *Biochim Biophys Acta* 1421: 19–31, 1999.
24. May JM, Qu ZC, and Cobb CE. Extracellular reduction of the ascorbate free radical by human erythrocytes. *Biochem Biophys Res Commun* 267: 118–123, 2000.
25. May JM, Qu ZC, and Cobb CE. Recycling of the ascorbate free radical by human erythrocyte membranes. *Free Radic Biol Med* 31: 117–124, 2001.
26. May JM, Qu ZC, and Cobb CE. Human erythrocyte recycling of ascorbic acid: relative contributions from the ascorbate free radical and dehydroascorbic acid. *J Biol Chem* 279: 14975–14982, 2004.
27. May JM, Qu ZC, and Morrow JD. Mechanisms of ascorbic acid recycling in human erythrocytes. *Biochim Biophys Acta* 1528: 159–166, 2001.
28. May JM, Qu ZC, Morrow JD, and Cobb CE. Ascorbate-dependent protection of human erythrocytes against oxidant stress generated by extracellular diazobenzene sulfonate. *Biochem Pharmacol* 60: 47–53, 2000.
29. May JM, Qu ZC, and Whitesell RR. Ascorbate is the major electron donor for a transmembrane oxidoreductase of human erythrocytes. *Biochim Biophys Acta* 1238: 127–136, 1995.
30. May JM, Qu ZC, Whitesell RR, and Cobb CE. Ascorbate recycling in human erythrocytes: role of GSH in reducing dehydroascorbate. *Free Radic Biol Med* 20: 543–551, 1996.
31. Meister A and Anderson ME. Glutathione. *Annu Rev Biochem* 52: 711–760, 1983.
32. Messana JM, Cieslinski DA, O'Connor RP, and Humes HD. Glutathione protects against exogenous oxidant injury to rabbit renal proximal tubules. *Am J Physiol* 255: F874–F884, 1988.
33. Mishra RK and Passow H. Induction of intracellular ATP synthesis by extracellular ferricyanide in human red blood cells. *J Membr Biol* 1: 214–224, 1969.
34. Momsen G. Effect of increasing the intracellular ratio of NADH to NAD^+ on human erythrocyte metabolism: new estimation of the turnover through the phosphoglycerate shunt. *Arch Biochem Biophys* 210: 160–166, 1981.
35. Pendergrass JA, Srinivasan V, Clark EP, and Kumar KS. Glutathione redox status in the human cell line, A549, following intracellular glutathione depletion and extracellular glutathione addition. *Tox Subst Mech* 18: 11–20, 1999.
36. Perego P, Gatti L, Carenini N, Dal Bo L, and Zunino F. Apoptosis induced by extracellular glutathione is mediated by H_2O_2 production and DNA damage. *Int J Cancer* 87: 343–348, 2000.
37. Rae CD, Berners-Price SJ, Bulliman BT, and Kuchel PW. Kinetic analysis of the human erythrocyte glyoxalase system using ^{1}H NMR and a computer model. *Eur J Biochem* 193: 83–90, 1990.
38. Russell J, Spickett C, Reglinski J, Smith W, McMurray J, and Abdullah I. Alteration of the erythrocyte glutathione redox balance by *N*-acetylcysteine, captopril and exogenous glutathione. *FEBS Lett* 347: 215–220, 1994.
39. Schipfer W, Neophytou B, Trobisch R, Groiss O and Goldenberg H. Reduction of extracellular potassium ferricyanide by transmembrane NADH: (acceptor) oxidoreductase of human erythrocytes. *Int J Biochem* 17: 819–823, 1985.
40. Tan AS and Berridge MV. Superoxide produced by activated neutrophils efficiently reduces the tetrazolium salt WST-1 to produce a soluble formazan: a simple colorimetric assay for measuring respiratory burst activation and for screening anti-inflammatory agents. *J Immunol Methods* 238: 59–68, 2000.
41. Thorburn DR and Kuchel PW. Regulation of the human-erythrocyte hexose-monophosphate shunt under conditions of oxidative stress: a study using NMR spectroscopy, a kinetic isotope effect, a reconstituted system and computer simulation. *Eur J Biochem* 150: 371–386, 1985.
42. Tietze F. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. *Anal Biochem* 27: 502–522, 1969.
43. Tilton WM, Seaman C, Carriero D, and Piomelli S. Regulation of glycolysis in the erythrocyte: role of the lactate/pyruvate and NAD/NADH ratios. *J Lab Clin Med* 118: 146–152, 1991.
44. Van Duijn MM, Tijssen K, VanSteveninck J, Van der Broek P, and Van der Zee J. Erythrocytes reduce extracellular ascorbate free radicals using intracellular ascorbate as an electron donor. *J Biol Chem* 275: 27720–27725, 2000.

45. Van Duijn MM, Van der Zee J, and Van den Broek PJ. The ascorbate-driven reduction of extracellular ascorbate free radical by the erythrocyte is an electrogenic process. *FEBS Lett* 491: 67–70, 2001.
46. Van Duijn MM, Vanderzee J, Vansteveninck J, and Vandenbroek PJA. Ascorbate stimulates ferricyanide reduction in HL-60 cells through a mechanism distinct from the NADH-dependent plasma membrane reductase. *J Biol Chem* 273: 13415–13420, 1998.
47. Villalba JM, Navarro F, Cordoba F, Serrano A, Arroyo A, Crane FL, and Navas P. Coenzyme Q reductase from liver plasma membrane: purification and role in trans-plasma-membrane electron transport. *Proc Natl Acad Sci U S A* 92: 4887–4891, 1995.
48. Zamudio I and Canessa M. Nicotinamide-adenine dinucleotide dehydrogenase activity of human erythrocyte membranes. *Biochim Biophys Acta* 120: 165–169, 1966.
49. Zerez CR, Lachant NA, and Tanaka KR. Impaired erythrocyte methemoglobin reduction in sickle cell disease: dependence of methemoglobin reduction on reduced nicotinamide adenine dinucleotide content. *Blood* 76: 1008–1014, 1990.

Address reprint requests to:
Prof. Philip Kuchel
School of Molecular and Microbial Biosciences
University of Sydney
NSW, Australia

E-mail: p.kuchel@mmb.usyd.edu.au

Date of first submission to ARS Central, February 7, 2006; date of acceptance, February 19, 2006.

This article has been cited by:

1. Hans Löw, Frederick L. Crane, D. James Morré. 2012. Putting together a plasma membrane NADH oxidase: A tale of three laboratories. *The International Journal of Biochemistry & Cell Biology* **44**:11, 1834-1838. [CrossRef]
2. Eri Ogawa, Nobuya Hishiyama. 2012. Japanese Shiba dogs possessing erythrocytes with high Glut-1 activity and high ascorbic acid recycling capacity. *Comparative Clinical Pathology* **21**:5, 639-644. [CrossRef]
3. Carol S. JohnstonVitamin C 248-260. [CrossRef]
4. Domenico Del Principe , Luciana Avigliano , Isabella Savini , Maria Valeria Catani . 2011. Trans-Plasma Membrane Electron Transport in Mammals: Functional Significance in Health and Disease. *Antioxidants & Redox Signaling* **14**:11, 2289-2318. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
5. Pawan Kumar Maurya, Siya Prakash. 2011. Intracellular uptake of (-)epicatechin by human erythrocytes as a function of human age. *Phytotherapy Research* **25**:6, 944-946. [CrossRef]
6. Syed Ibrahim Rizvi, Neeti Srivastava. 2010. Erythrocyte plasma membrane redox system in first degree relatives of type 2 diabetic patients. *International Journal of Diabetes Mellitus* **2**:2, 119-121. [CrossRef]
7. Darius J.R. Lane, Stephen R. Robinson, Hania Czerwinska, Alfons Lawen. 2010. A role for Na + /H + exchangers and intracellular pH in regulating vitamin C-driven electron transport across the plasma membrane. *Biochemical Journal* **428**:2, 191-200. [CrossRef]
8. Syed Ibrahim Rizvi , Kanti Bhooshan Pandey , Rashmi Jha , Pawan Kumar Maurya . 2009. Ascorbate Recycling by Erythrocytes During Aging in Humans. *Rejuvenation Research* **12**:1, 3-6. [Abstract] [Full Text PDF] [Full Text PDF with Links]
9. Darius J.R. Lane, Alfons Lawen. 2008. A highly sensitive colorimetric microplate ferrocyanide assay applied to ascorbate-stimulated transplasma membrane ferricyanide reduction and mitochondrial succinate oxidation. *Analytical Biochemistry* **373**:2, 287-295. [CrossRef]
10. Darius J. R. Lane, Alfons Lawen. 2008. Transplasma membrane electron transport comes in two flavors. *BioFactors* **34**:3, 191-200. [CrossRef]