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# OXIDOREDUCTION *at the* PLASMA MEMBRANE: Relation to Growth and Transport

## Volume I

Frederick L. Crane
D. James Morré
Hans E. Löw

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## Volume I
## Animals


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# PREFACE

The objective of this volume is to present a comprehensive coverage of the evidence that oxidoreductase enzymes in the plasma membranes of animal cells play an important role in control of cell growth and to further examine how the oxidoreductase enzymes may influence control of membrane transport. Although isolated experiments in plasma membrane electron transport can be found as far back as 1929, it is only in the last 15 years that a systematic study has begun on the clear determination of the widespread existence of unique plasma membrane oxidoreductase enzymes. As evidence for the presence of plasma membrane redox enzymes solidified, experiments were designed to show potential roles in growth, development, and membrane transport.

At least twenty laboratories around the world have been involved in current studies of plasma membrane oxidoreductase enzymes in animal cells, and even more have worked with plants. The results of these investigations are mostly scattered throughout the literature except for two recent books that contained reports presented at meetings. As a result, it is difficult to evaluate the scope of research which supports the general concept of plasma membrane oxidoreductase enzymes and the evidence which is developing for their contribution to cell function. We hope that these volumes will help to consolidate this basic information. We also expect that these books will serve to emphasize that much more research is needed to understand the inner workings of the electron transport enzymes, how they help to control the cell cycle, where they contribute to transport systems, and their ubiquitous occurrence in living cells. Beyond basic understanding, lies the practical significance which may be revealed in changes in these enzymes in tumor cells and control of the enzymes by antitumor and antimalarial drugs and neuromodulators.

The chapters are organized to present first the basic observation of plasma membrane oxidoreductase activity and the unique properties of these enzyme activities, especially with respect to selective inhibitor and hormone response. The next chapters consider the stimulation of cell growth by transplasma membrane electron transport. This discussion of growth control leads to examination of transmembrane iron and ferric transferrin reduction and its relation to transferrin receptor involvement in growth control. The last chapters consider the effects of plasma membrane electron transport on physiological functions of cells which could be involved in growth control and the relation of the redox system to antitumor drug action and response to neurotransmitters.

The material presented in this book is only half of the story of plasma membrane oxidoreductases. Enzymes of similar character are also present in plant plasma membranes. A discussion of these enzymes and their relation to plant growth will be presented in a second volume.

We very much appreciate the efforts of the contributors who have taken the time to prepare chapters which present a comprehensive view of the subject. We also thank Janet Hollister for skillful help in assembling the final copy and Annika Lindgren and Monica Isaksson for preparation of figure copy.

**Frederick L. Crane**
**D. James Morré**
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Dr. Crane obtained his training at the University of Michigan, Ann Arbor, receiving the B.S. degree in chemistry in 1950, followed by the M.S. in 1951, and the Ph.D. in 1953 in botany. From 1953 to 1957 he was a postdoctoral trainee at the Enzyme Institute, University of Wisconsin, where he was Assistant Professor from 1957. In 1959 he was appointed Assistant Professor in the Department of Chemistry, University of Texas, Austin. In 1960, he moved as Associate Professor to the Biology Department of Purdue where he assumed his present position in 1962. He has had visiting appointments at Stockholm University, 1963 to 1964, and at the John Curtin School of Medical Research, Australian National University, Canberra, in 1970/1971 and 1979/1980.

Dr. Crane is a member of the American Society for Biological Chemistry and Molecular Biology, the American Society for Cell Biology, the American Society of Plant Physiologists, the Plant Growth Regulator Society of America, and the Indiana Academy of Science.

In 1961 he was recipient of the American Chemical Society Eli Lilly Medal in Biochemistry. He was awarded a Silver Medal in Biochemistry at the University of Bologna and an Honorary Doctor of Medicine Degree by the Karolinska Institute in Stockholm in 1989.

Dr. Crane is recipient of a Research Career Award from the National Institutes of Health and has served on Research Review Panels at the National Science Foundation and the National Institutes of Health. He was co-chairman of a meeting on Plasmalemma Redox Function in Plants at the 1987 Botanical Congress in Berlin, and was Director of a NATO Advanced Research Workshop on Plasma Membrane Oxidoreductases in Control of Animal and Plant Growth in Cordoba in 1988.

Dr. Crane has served on the editorial board of *Biochimica et Biophysica Acta* and is Review Editor of the *Journal of Bioenergetics and Biomembranes*. He is an author of more than 350 papers and coeditor of two books. His current research interest is the nature and function of plasma membrane oxidoreductases.

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Dr. Löw is a member of several Swedish and foreign learned societies and was co-organizer of several symposia related to plasma membrane redox and growth factor action. He is an author of about 200 scientific papers and co-editor of two books. His current research interest includes apart from the mechanism and function of the plasma membrane oxidoreductase reaction, the mode of action of insulin and growth factors with special reference to their function in the diabetic state.

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Chapter 1

# HISTORICAL PERSPECTIVE

**F. L. Crane, H. Löw, and D. J. Morré**

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# I. RECOGNITION OF PLASMA MEMBRANE OXIDOREDUCTASES

The comprehensive study of oxidoreduction reactions in the plasma membrane has been a recent development. It is known now that there are oxidoreductase enzymes on the inner face, the exterior face, and crossing the membrane. Evidence is growing for significant functions for these enzymes.[1-9] Preliminary observations of these oxidoreductases can be traced back to the 1920s, but many of these early observations seem to have faded out of sight before they could stimulate a major investigation into the nature and significance of the oxidoreductases in the plasma membrane.

There are six major lines of investigation which led into the concepts of redox function in the plasma membrane and its functional significance (Table 1). These areas include:

1. Studies of oxidation-reduction reactions of whole cells with impermeable oxidants indicating electron transfer across the plasma membrane: the logical development of this observation would have been study of oxidoreductases in isolated plasma membrane but this was difficult because highly purified plasma membranes were hard to prepare and the oxidoreductase activity in contaminating mitochondrial or endoplastic reticulum membranes overwhelmed any plasma membrance activity. The mammalian erythrocyte membrane was a special case because the absence of internal membranes precluded contamination of plasma membrane preparations by other membranes. This led to early studies of redox functions with these membranes, but the primary emphasis was on known extensions of endoplasmic redox function such as NADH cytochrome c reductase[10] and methemoglobin reduction,[11,12] which represents a useful function of this enzyme in erythrocytes.
2. Stimulation of cell growth by external oxidants: although Brooks' studies in 1947[13] could have opened up this area of research, there seems to have been no further development until evidence for transplasmalemma electron transport stimulated a new study by Ellem and Kay in 1983[14] on using ferricyanide as an external oxidant to stimulate growth.
3. The study of salt respiration in plants led to proposals that plasma membrane redox enzymes could provide energy for ion uptake directly without mediation by ATP.[15] The impetus for these studies was decreased by development of evidence for ATPases to drive transport. New interest developed with evidence for redox effects on amino acid transport. Suggested relations between redox and gastric acid secretion have always been in the background.
4. Evidence that the pigments responsible for response of plants to blue light are located in the plasma membrane:[16] this reaction has specific control function for plant and fungal cells. Questions about plasma membrane purity led to debate about location in the cell.
5. The respiratory burst of leucocytes with consequent hydrogen peroxide production to kill engulfed bacteria led to investigations of the oxidase reaction and evidence for its location in the plasma membrane.[17]
6. External peroxide production in the wall of plant cells has long been related to lignin formation in the cell wall. Peroxidases associated with this peroxidation are bound to the surface of the plasma membrane.[18]

The studies by Voegtlin, Johnson, and Dyer in 1925 were designed to examine a relation between the protoplasmic redox state and cancer,[19] a subject which later attracted others such as Warburg.[20] In their studies, they used both permeable and impermeable oxidoreduction indicator dyes to assess the reduction rate by normal cells and tumor cells. They used a very

## TABLE 1
## Historical Developments in Plasma Membrane Redox

| Dates | Reference events | Tissues, cells | Membranes | Cell division |
|---|---|---|---|---|
| 1920 | 1923 — 1933, 1948, Keilin, Warburg — respiratory system<br>Green, Lehninger — Mitochrondria as site of energy transfer | 1925, Voegtlin et al. — impermeable dye reduction<br>1925, Barron — transmembrane electron transport<br>1954, Manyai and Szekely — impermeable ferricyanide introduced as external oxidant | | 1947, Brooks — dyes increase cell division in eggs |
| 1960 | 1965, P. Mitchell — chemiosmotic hypothesis | 1965, Dormandy and Zarday — ferricyanide reduction and $H^+$ release by erthrocytes<br>1969, Mishra and Passow — ferricyanide and erythrocyte | 1964, Emmelot — NADH cyt c reductase in erythrocyte PM.<br>1966, Wallach and Kant — Ehrlich ascites ferricyanide reductase<br>1966, Zamudio and Canessa — NADH ferricyanide reductase in erythrocyte<br>1967, Vassilez et al. — NADH dehydrogenase in liver PM | |
| 1970 | 1971, Morré — membrane flow | 1972, Arese et al. — NAD(P)H oxidized by ferricyanide in erythrocytes<br>1977, Mukherjee and Lynn — NADPH oxidase in plasma membrane oxidizes SH<br>1978, Morré et al. — cytochemistry, Hatchett Brown<br>1977, Garcia-Sancho et al. — redox-driven amino acid transport | 1970, Fischer — squid axon membrane<br>1973, Masuda et al. — NADH-cyt c in liver plasma membrane<br>1973, Frantz, Vigil et al. — liver cell plasma membrane; ferricyanide reduction<br>1976, Löw and Crane — inhibition and hormones; liver plasma membrane<br>1977, Gayda — NADH oxidase<br>1978, Wang and Alaupovic — isolated dehydrogenase<br>1979, Löw et al. — oriented dehydrogenase<br>1979, Huang et al. — gel electrophoresis of plasma membrane enzymes | |

## TABLE 1 (continued)
## Historical Developments in Plasma Membrane Redox

| Dates | Reference events | Tissues, cells | Membranes | Cell division |
|---|---|---|---|---|
| 1980 | 1982, Albertsson et al. — phase separation for plasma membrane purification | 1981, Clark et al. — ferricyanide reduction by liver cells and perfused liver<br>1981, Powis et al. — indigo-disulfonate reduction; AKR cells; transformation<br>1984, Sun — $H^+$ release with ferricyanide reduction<br>1986, Navas et al. — NADH oxidized by ferricyanide; HeLa | 1980, Goldenberg — SDHA reductase in PM<br>1983, Morley et al. — differic transferrin reduction | 1980, Barnes and Sato — transferrin required for growth<br>1983, Kay and Ellem — ferricyanide + cell growth<br>1984, Sun et al. — ferricyanide and HeLa growth<br>1985, Sun et al. — redox dyes and growth of HeLa |

| Dates | Plants: plasma membrane | Blue light effects in plasma membrane | Defense and peroxide formation | External peroxidase and dehydrogenase |
|---|---|---|---|---|
| 1920 | 1945, Lundegardh — salt respiration<br>1951, Conway — redox drive in yeast | | 1954, Morton — xanthine oxidase in milk fat membrane | 1957, Crane — cauliflower wall fraction has CN-insensitive NADH oxidase<br>1959, Forti et al. — NADH oxidase in heavy pea stem particles |
| 1960 | 1968, Roberston — redox transport | | | |
| 1970 | 1977, Polevoi and Salamatova — ferricyanide inhibits auxin-induced $O_2$ uptake<br>1977, Chaney et al. — transmembrane iron reduction | 1974, Poff and Bulter — blue light effect on plant growth<br>1977, Brain<br>1977, Jesaitis — blue light reduction reactions in PM; flavin and cytochrome | 1970, Keenan — milk fat membrane is plasma membrane<br>1975, Briggs et al. — NADH oxidase in plasma membrane neutrophils $O2^-$<br>1979, Dewald et al. — NADPH oxidase<br>1979, Borregaard et al. — redox components and granulomatous disease | 1976, Elstner and Heupel — NADH oxidation by wall peroxidase<br>1977, Gross — peroxide in plant cell wall<br>1979, Löw et al. — NADH dehydrogenase outside erythrocyte |

| | | | | |
|---|---|---|---|---|
| 1980 | 1981, Novak and Ivankina — PM redox and membrane potential<br>1981, Craig and Crane — carrot ferricyanide reduction<br>1984, Sijmons et al. — NADPH oxidation in cytosol<br>1985, Bienfait — the Turbo reductase<br>1985, Barr et al. — CN-insensitive NADH oxidase in plant plasma membrane | 1981, Widell et al. — blue light effects in pure plasma membrane<br>1985, Kjellbom et al. — P450 | 1980, Green et al. — NADPH oxidase is transmembrane<br>1981, Cross et al. — Low potential cyt b | 1981, Cherry — NADH dehydrogenase outside tumor cell<br>1982, Lin — external oxidase on plant cells<br>1982, Mader and Amberg-Fischer — external oxidase is peroxidase in plants<br>1987, Morré et al. — hormone controlled oxidase outside<br>1985, Barr et al. — cyanide-insensitive NADH oxidase is not peroxidase |

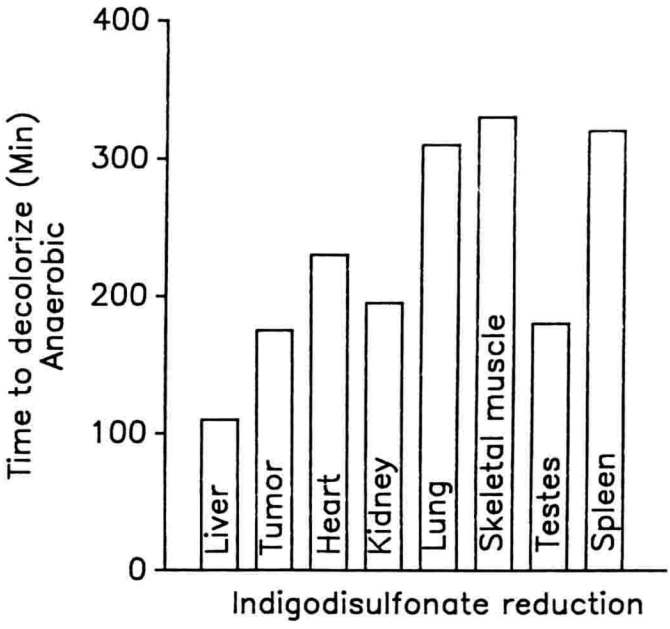


FIGURE 1. Reduction rate of indigo disulfonate exposed to rat tissue rats showing the time taken for the rat to decolorize. (Calculated from data in Reference 19).

simple experimental procedure to determine redox capacity. The time it took to decolorize the dye with a tissue suspension under anaerobiosis was determined and expressed as time to decolorize: in general, the higher the redox potential of the dye, the faster the rate of decolorization (reduction). Both permeable and impermeable dyes were reduced by the cells from all tissues, but the rate of reduction was clearly different from tissue to tissue. In looking at overall dye reduction, including both permeable and impermeable dyes, there was no apparent difference between Flexnor-Jobling tumor tissue and normal liver tissue. If the results of these experiments are considered on the basis that the impermeable dyes measure transplasma membrane electron transport whereas permeable dyes are reduced by many redox systems inside the cell, interesting observations relating to plasma membrane redox are possible. With impermeable indigo disulfonate the reduction rate by Flexnor-Jobling tumor can be half the rate of the one observed with liver (or two times faster than lung). The fact that the impermeable dyes are reduced is in itself good evidence for some type of electron transfer across the plasma membrane and the reduction of indigo disulfonate indicates electron transfer at a relatively low potential ($-125$ mV) (Figure 1).

Experiments to determine the toxicity of dyes by injecting them into rats gave further evidence for the difference between permeable and impermeable dyes. The impermeable dyes, even though reduced, show very little lethality. They do produce respiratory problems. The permeable dyes were rapidly lethal. This would be consistent with rapid oxidation of cytosolic reductants such as NADH, NADPH, and glutathione, as well as interference with mitochondrial ATP production by the permeable dyes. The impermeable dyes would give a more selective oxidation of cytosolic reductants by electron transfer across the plasma membrane and would not interfere with mitochondrial ATP production by short circuiting the electron transport chain.

The comparison of effects of permeable and impermeable dyes was more fully developed in experiments on starfish eggs by Barron and Hoffman in 1927.[21] They used autooxidizable permeable and nonpermeable dyes to test for effects on oxygen uptake. The permeable dyes

stimulate oxygen uptake at low redox potential which is consistent with electron donation by cytosolic redox compounds like NADH or glutathione with redox potentials in the range of −320 to −280 mV. Increased oxygen uptake was only found with high potential impermeable dyes. Gallocyanine (59 mV) gave increased oxygen uptake whereas indigo-disulfonate (−125 mV) had no effect. They came to the clear conclusion that impermeable dye reduction would involve a transplasma membrane electron transport with a component of midpoint potential above −125 mV at the cell surface. It should be noted that restriction of reduction to a redox potential that high would preclude direct reduction by leakage of glutathione or NADH out of the cell, but not ascorbate (+59 mV). The difference between indigo-disulfonate reduction by rat liver cells and lack of reduction by sea urchin eggs may also indicate a difference between external reduction sites in different organisms.

The cytochromes had been rediscovered in 1923—1933 by Keilin[22] and Warburg[23] as components of the major cyanide sensitive respiratory chain. The role of ATP in energy transfer reactions was developed by Lipmann[24] in 1939—1941 and the Krebs cycle was proposed in 1937.[25] Only in 1948 was the combined respiratory system and Krebs cycle, or cyclophorase, proposed by Green[26] and identified with mitochondria by Kennedy and Lehninger.[27] These studies focused the major attention in respiratory activity and especially energy-coupled respiratory activity on the mitochondria. The primary mechanism for energy transfer from both glycolysis and mitochondrial oxidation was recognized as ATP. There was thus no obvious basis to look for redox systems in the plasma membrane to energize any function. The presence of significant membrane-bound redox function not involved in energy transfer, such as the cytochrome b fatty acyl desaturase system and the detoxification system with cytochrome P450, was not developed until 1960s.[28]

In 1945, Lundegardh[29] proposed a special cytochrome system in plant cells that would directly drive ion uptake. To provide direct coupling between the electron transfer and ion transport, this redox system should have been located in the plasma membrane (Figure 2). Without isolated plasma membranes, the identity of the cytochrome changes which responded to addition of salts was difficult to establish.[30] The well-known salt respiration could not clearly be differentiated from mitrochondrial respiration. In 1948, Conway and Brady[31] proposed a plasma membrane redox system as a basis for gastric secretion which would be directly coupled to proton pumping. During this period, until 1968, Robertson[32] also investigated salt respiration and developed further evidence of its unique properties, but a clear definition of a plasma membrane redox system was not possible, primarily because isolation of sufficiently purified plasma membranes was not possible.

In 1954, Manyai and Szekely[33] showed that ferricyanide, which was impermeable to erythrocytes, caused an increase in ATP inside erythrocytes. This implied a possible redox function in the plasma membrane coupled to ATP formation since these cells lack mitochondria. Later studies indicated that the increase in ATP could come from activation of glycolysis by increase in NAD or NADP by oxidation of NADH or NADPH. The oxidation of NADH or NADPH would provide an acceptor to activate glycolytic activity.[34] Only 10 years later, Dormandy and Zarday[35] showed that erythrocytes reduce ferricyanide. They also showed that ferricyanide reduction was directed to proton release, and that this activity was stimulated by insulin. It was at this time (1965) that Mitchell proposed the chemiosmotic hypothesis as the basis for coupling electron transport and transmembrane proton movement to ATP formation in mitochondria[36] (Figure 3). The Mitchell hypothesis was cited by Mishra and Passow in their discussion of possible functions for the plasma membrane ferricyanide reductase when they demonstrated ferricyanide reduction by erythrocytes and associated ATP formation in 1969.[34]

The clearest demonstration of plasma membrane NADH dehydrogenase activity was made by Zamudio and Canessa[37] with erythrocyte membranes. They showed very active NADH ferricyanide reductase activity. Later, Zamudio et al.[38] showed that nitroblue tet-