Catalase, Cu/Zn- Superoxide Dismutase, Glutathione Peroxidase: Their Relationship to Oxygen Utilization in Cellular Physiology, Clinical and Subclinical Disease, Nutrition and Trace Element Utilization in Livestock

Robert T. Coffey, *D.V.M.*
Shiloh-Livestock Veterinary Services
RR 1, Box 362
Newton, Iowa 50208

Introduction

There is a definite inter-relationship between cellular physiology and the incidence of subclinical and clinical disease. Alterations in normal cellular functions can predispose animals to a wide variety of infectious and non-infectious situations. An understanding of the inherent protective mechanisms of the individual cell and how these alterations manifest themselves in various physical symptoms, the influence of environment, and the physiological impact of adequate nutrition, aids in the development of sound preventative medicine programs. Catalase, Cu/Zn-superoxide dismutase, and glutathione peroxidase are three red blood cell enzymes that have a protective role in the animal and serve as a biological monitor of the physiological and nutritional state of the animal.

Oxygen Utilization and Cellular Physiology

Oxygen is an essential component in all phases of mammalian cellular physiology. Every organ and organ system incorporates the use of oxygen, directly or indirectly, to fulfill a specified function. There is a definitive feedback mechanism within normal physiology that regulates cellular metabolic processes, immune response, cellular proliferation, hormone production, and adaptibility of the species to its environment (18, 21). When there is a negative alteration of cellular physiology, for whatever the reason, the system becomes vulnerable to a breakdown, and invasion by outside factors leading to clinical or subclinical disease, infectious or non-infectious disease, or self-destruction is imminent (Figure 1) (19).

An understanding of the mechanism by which oxygen is incorporated into the basic physiological processes of cellular metabolism lends itself to developing a better insight into the pathogenesis of disease (21). The idea of "oxygen toxicity" has been around for many years (22). But only recently, has there been any emphasis placed on the clinical impact of oxygen radical physiology in veterinary medicine.

"Oxygen toxicity refers to the superoxide theory of oxygen toxicity. The superoxide radical, O_2^-, is formed during various metabolic processes, many of which are considered normal. Liver cells, muscle cells, leukocytes, erythrocytes, aerobic bacteria, any cell that undergoes oxidative cellular metabolism, all form oxygen radicals during normal metabolic processes (21, 23). These oxygen radicals are converted to hydrogen peroxide (Reaction 1) or the more deadly hydroxide radical (Reaction 2):

Reaction 1: $O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$

Reaction 2: $H_2O_2 + O_2^- \rightarrow {}^{\cdot}OH + OH^- + O_2$

When these reaction become uncontrolled or the animal loses the ability to regulate these reactions, there are changes in cellular physiology that become detrimental to individual cells, organ systems, or the entire animal. Some of these changes include generalized tissue destruction, lameness and joint inflammation, DNA degradation, lipid peroxidation, altered immune function, and inactivation of important cellular enzymes (17, 23).

Catalase, Cu/Zn-SOD, and GPx are three of the primary enzymes that control superoxide radical anions and hydrogen peroxide accumulation. (13,30) Figure 2 is a schematic representation of the inter-relationship of these three enzymes.

Cu-Zn SOD catalyzes the following reaction: (12)

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

Catalase catalyzes the following reaction: (9)

$$H_2O_2 + H_2O_2 \rightarrow 2H_2O + O_2$$

GSH-Px catalyzes the GSH-dependent reduction of hydroperoxides as follows: (20)

$$2GSH + ROOH \rightarrow GSSG + ROH + H_2O$$

FIGURE 1. Livestock Production

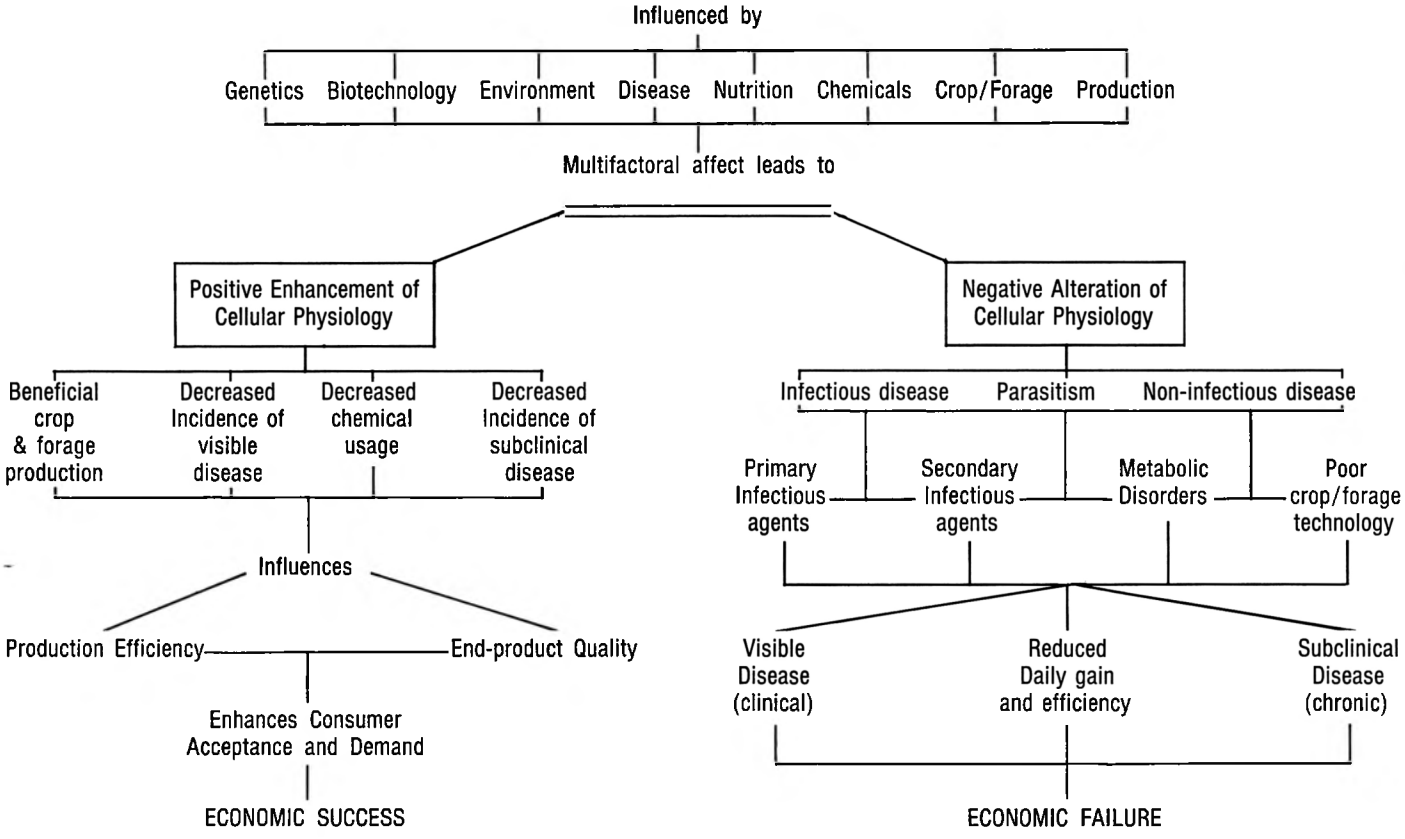
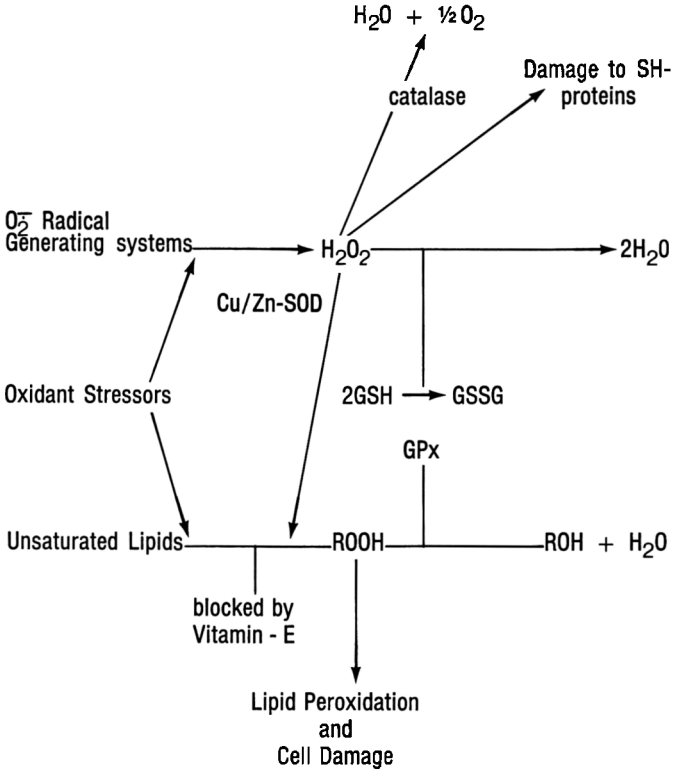


FIGURE 2. Summary Schematic



Catalase is a large molecular weight enzyme that contains four heme groups per molecule (9). Review of the metabolic pathway of heme formation illustrates the incorporation of iron into this protein as well as the functional role copper has in the formation of protoporphyrinogen III, the predecessor of the heme molecule (25). Since catalase contains the heme molecule, a basic understanding of iron metabolism is essential to realize the importance of this enzyme and those heme enzymes of the cytochrome system and the role they play in cellular oxygen utilization (4). It is easy to see the importance of catalase and how other metabolic pathways affect its formation and activity. The enzyme is commonplace in most cells of the body.

Cu/Zn-SOD is a metalloenzyme that undergoes a reduction-oxidation cycle with the oxygen radical with the net result of dismutation of the superoxide radical to hydrogen peroxide and oxygen [see Reaction 1] (14). The catalytic metals for this primary activity are copper and zinc, but the enzyme has a manganese form [Mn-SOD] that occurs in cellular mitochondria and an iron form [Fe-SOD] known to be active in certain bacterial processes (14). The apoenzyme (without copper) is virtually inactive in the animal. Therefore part of the pathogenesis of subclinical copper deficiency includes suppression of the beneficial effects of Cu/Zn-SOD. This enzyme activity can be suppressed by rapid accumulation of hydrogen peroxide (12). Copper amino acid complexes (chelates) are capable of catalysing the dismutation of the reactive oxygen radical in a similar fashion as Cy/Zn-SOD (23, 24).

Glutathione Peroxidase (GPx) is a selenium dependent enzyme that contains four moles of selenium per mole of GPx. Its role as an initial detoxifier of hydrogen peroxide is considered very important as well as the role it plays in the cellular protection mechanism against oxygen toxicity (27). In this regard it has a synergistic role with catalase. GPx determination is also recognized as a useful indicator of functional selenium activity. The substrate of GPx is glutathione)GSH). There is a requirement for ATP and Mg^{++} in the reactions of the GSH-synthtase system in the production of the tripeptide GSH and it also has a vital function in maintaining the integrity of the red blood cells (26, 35).

Oxygen Radicals in Disease

Not all aspects of oxygen radical production are detrimental. One of the most useful purposes of oxygen radical, peroxide, and hydroxide radical production is the role they play in the immune response when polymorphonuclear leukocytes engulf bacteria or immune complexes and destroy them. As oxygen radicals increase systemically it will initiate a more active immune response, but if left uncontrolled it can be devastating to the animal causing massive cellular destruction (5, 23, 33). This same type of immune hypersensitivity may be initiated when artificial antigens are introduced into the animal, such as with bacterins, vaccines, or even parasitisms (34).

An increase in oxygen radical production can be initiated with a release of adrenaline which is the normal physiologic response to stress. In veterinary medicine "stress" is recognized as one of the primary factors predisposing animals to disease. This same increase in oxygen radical production can be artificially induced with the administration of cortisone (23). A valid argument at this point is: Does a rapid build-up in oxygen radicals in-vivo provide a micro-aerophilic environment condusive to pathogen proliferation?

The diet of an animal can have a decisive impact on the incidence of oxygen toxicity. Heavy metal intake, iron salts, fatty acid accumulation, trace element deficiencies, vitamin deficiencies, rapid carbohydrate metabolism, and hypoproteinemia can all lead to the production of oxygen radicals and their detrimental effects.

A rapidly expanding and exciting area of research involves the understanding of the cyclic nucleotides, cAMP and cGMP, and the role they play in the regulation of cellular physiology. It is known that an increase in oxygen radical production will stimulate the phagocytic activity of leukocytes by stimulating cGMP (5). Lipid peroxides and hydrogen peroxide can stimulate prostaglandin synthesis which promotes the conversion of adenylate cyclase to cAMP (6,28). If left uncontrolled, prostaglandin production will potentiate endotoxic enteric disease as well as circulatory disturbances in the animal (kaneko). Almost all cellular activity is controlled by the ratio of cGMP to cAMP. If the relative level of cAMP is increased or that of cGMP is decreased then cellular activities tend to be inhibited. In contrast, if cAMP is lowered or cGMP is increased then overall cellular activity is going to be enhanced. Many of the physiological cellular mechanisms that regulate the immune response do so by a direct or indirect regualtion of the cAMP/cGMP ratio within cells (6, 38).

Any inflammatory process that would increase cellular oxidative activities (the production of oxygen radicals) would lead to the increased production of hydrogen peroxide, increasing catalase, Cu/Zn-SOD, and GPx activity (or demand for active enzyme). Chastain et al have shown Cu/Zn SOD activity to be suppressed in cattle affected with the bovine respiratory disease complex which originated in areas where they grazed fescue grown on selenium deficient soils (8, 37).

Another detrimental aspect of this uncontrolled activity is that of hydrogen peroxide on the primary storage form of the iron-protein complex called ferritin. Hydrogen peroxide will rapidly oxidize ferritin to the less labile form of storage iron known as hemosiderin which is a poor source

of iron for heme production and erythropoiesis (30, 39). Many times this is manifested in various clinical diseases by an increased finding of hemosiderosis of the liver (as a subsequent finding in the bovine respiratory disease complex) and persistent low grade anemia (especially in sows with chronic lipid peroxidation). Lactoferrin production and ceruloplasmin activity may also be compromised (3, 22, 36).

Though most of the iron incorporated into the body is bound to a protein component, there are pro and con arguments that there is ample free serum iron capable of generating hydroxide radicals when there is excess oxygen radical production, as follows (23):

$$Fe^{2+} - \text{Complex} + H_2O_2 \rightarrow Fe^{3+} - \text{Complex} + {}^{\cdot}OH + OH^{-}$$

$$\text{Net: } O_2^{\cdot-} + H_2O_2 \xrightarrow[\text{Catalyst}]{Fe} O_2 \times {}^{\cdot}OH + OH^{-}$$

Adequate SOD activity would actually provide the hydrogen peroxide substrate for this reaction. A decrease in activity of catalase and glutathione peroxidase may be conducive to the propagation of these reactions. SOD has also been shown to have beneficial therapeutic purposes in inflammatory disease in human and veterinary medicine.

Nutrition and Oxygen Radicals

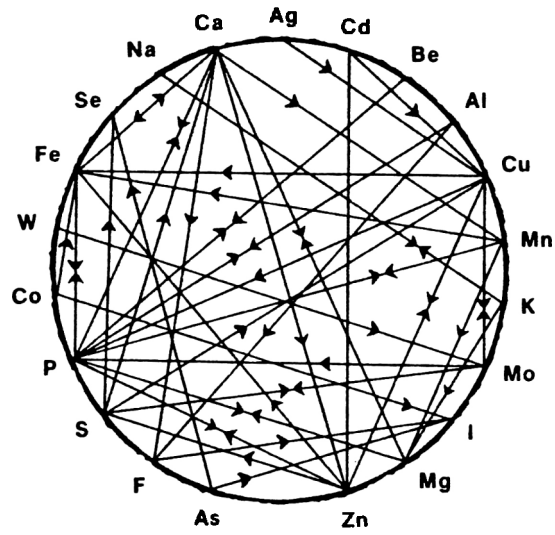
It has already been pointed out that nutrition plays an important role in proper cellular physiology. Review of Figure 1, illustrates the complexities involved in the multi-factorial causation of disease. Sometimes the most important but least understood factor is nutrition. All to often it is assumed that total ration intake in animals is adequate and properly balanced. In nutritional formulations the availability of nutrients is just as important as the quantity. Rations with high protein solubility or excess energy under stress conditions can be detrimental and lead to a rapid rise in oxygen radical production. Elemental salts may not be as available as chelated trace elements especially when there is excess heavy metal interference (2).

The most common nutritional problem that usually occurs is that of trace element deficiency. Copper, zinc, manganese, and selenium are of greatest concern and have a direct impact on the activity and formation of catalase, SOD, and GPx. Besides outright deficiencies, it is necessary to understand the inter-relationship of one mineral to another. Specific minerals may be present in perceived adequate amounts, but due to competition or interference it may not be biologically available. An example would be how molybedum directly ties up copper (40). As long as the molybedum level of intake is known, the total copper in the ration can be adjusted.

Another example is that of the heavier metals. Chronic excess aluminum intake (>500 ppm) is usually manifested by phosphorus deficiency but it also interferes with cellular phosphorylating mechanisms which decrease available serum ATP and thereby increasing adenosine monophosphate (AMP) (41). In combination with other heavy metals this could be potentiated, thereby suppressing cellular activities, including immune suppression. Vitamins also play an important part in cellular oxidative pathways as co-factors and directly blocking lipid peroxidation. Vitamin E is best known for its anti-oxidant activities (7, 16). As explained earlier these changes in cellular physiology can have a devastating impact on overall animal health, production and profitability (10, 15).

The interaction of various elements is graphically depicted in Dyer's mineral interaction chart, Figure 3 (1). The arrows indicate the antagonism that exists between minerals as they compete for absorption sites and/or carrier molecules in the digestive process of mineral utilization and uptake.

Figure 3. Dyer's Mineral Interaction Chart.

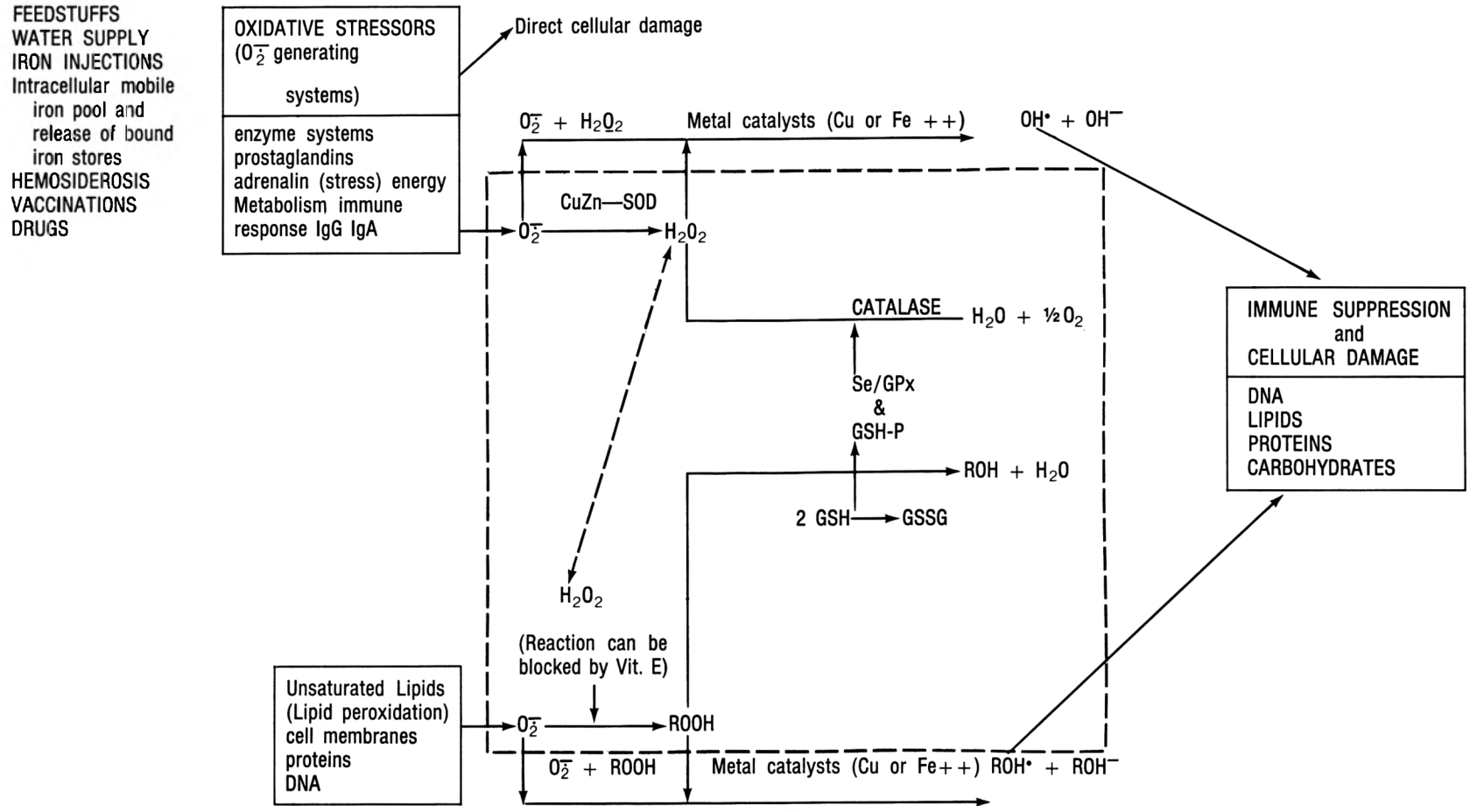


Summary

In this era of intense agricultural production practices and the vertical integration and specialization in livestock production, it is becoming vital that we understand these complex inter-relationships and the predisposition to disease. It may be the decisive factor between economic success and economic failure.

It becomes evident that Catalase, Cu/Zn-SOD, and GPx play a vital role in controlling many of the vital functions of cellular metabolism. The necessity of adequate trace element utilization and availability is essential in their formation and bioactivity. Copper and iron are important in catalase; copper and zinc in superoxide dismutase; and selenium in glutathione peroxidase. All too often in enzyme

SCHEMATIC OF THE PRIMARY CELLULAR OXIDATIVE REACTIONS



Within the dashed line (- - -) box are normal cellular defense mechanisims; when overwhelmed there is cellular damage and immune suppression.

and trace element studies it is difficult to obtain adequate respective samples from animals for proper determinations. These three enzymes are readily accessible within the red blood cell where their concentrations and activity can be determined.

As with any complex system there are many factors that influence the final interpretation of results. In livestock production and health it is necesasary to have a working knowledge of the differences that exist between breeds; the influence different management systems and environmental conditions have on cellular metabolism. Recording and analyzing clinical histories and conventional diagnostic data and its true significance is an art that comes with experience and knowledge. An applicable understanding of nutrition and nutritional interrelationships and the mechanisms of disease all have an impact on the final product (31, 32, 3).

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