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The effect of shock on blood oxidation-reduction potential

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Abstract. Oxidation-reduction (redox) potential measurements were made in the blood of rabbits subjected to hemorrhagic shock followed by treatment with a mild oxidizing agent (albumin). Control redox potential reading corrected for pH was -8.8 ± 1.3 millivolts (mV) in arterial blood (A) and -18.0 ± 2.0 mV in venous blood (V). This A-V difference indicated that hydrogen equivalents coming from muscle and other tissues were partially consumed in the lungs. A 20-mV drop on the V and a 13 mV on the A side was seen after shock. This did not fully return to control 2 h after return of the shed blood. Infusion of 2 g of albumin/kg/h raised the V redox potential to control, but it returned to untreated levels when the albumin was discontinued. The reductive load imposed on the animal by shock appeared to be large and not readily reversed by reperfusion or by the quantity of albumin given. Thus, it may be concluded that cellular respiration had not been adequately restored. This reductive load may impede recovery by suppression of cellular respiration and other cell and organ functions.
Key words. Hemorrhagic shock; oxidation-reduction potential; carbon electrode; denatured albumin; reductive load; oxygen radical challenge; reperfusion injury; thyroid function.

Acid-base balance, involving proton transfer, is described by the Bronsted theory and expressed by the Henderson-Hasselbach equation. It involves the hydrogen ion (H^+) (not to be confused with hydrogen), is measured as pH and is recognized as an important part of homeostasis in health and disease. In contrast, oxidation-reduction (redox) balance arising from ubiquitous biochemical redox reactions is equally important but less familiar. Redox balance involving electron transfer is described by the Nernst-Peters equation, and is measured as redox potentials or redox states. Hydrogen (electron, with proton) is transferred to carriers such as NADH, NADPH, sulfhydryls and others. This system, as it pertains to health and disease, has been reviewed by Shapiro [1].

According to Shapiro, redox balance, like acid-base balance, requires a normal range, with a rise defined as oxidosis and a fall defined as redosis. Like acidosis and alkalosis, oxidosis and redosis could be further classified as 'respiratory' or 'metabolic'. Oxygen availability is a respiratory determinant of redox states, so that an elevated pO_2 (hyperoxia) could give rise to respiratory oxidosis, and a low pO_2 (hypoxia) would result in respiratory redosis. Metabolic oxidosis is due to accumulation of oxidants, or depletion of reductants, and vice versa for metabolic redosis. These terms, proposed by Shapiro, have not been used commonly.

Redox potentials can be measured in blood and other body fluids at gold, plantinum or carbon electrodes against a reference (such as silver/silver chloride) electrode. A reflection of an overall tissue redox state is provided by the lactate/pyruvate ratio and 'excess lactate' as calculated by Huckabee [2]. Another expression of the general redox state in tissue may be seen in the ratio of oxidized to reduced pyridine nucleotides. Altered redox states occur in various pathological conditions, and

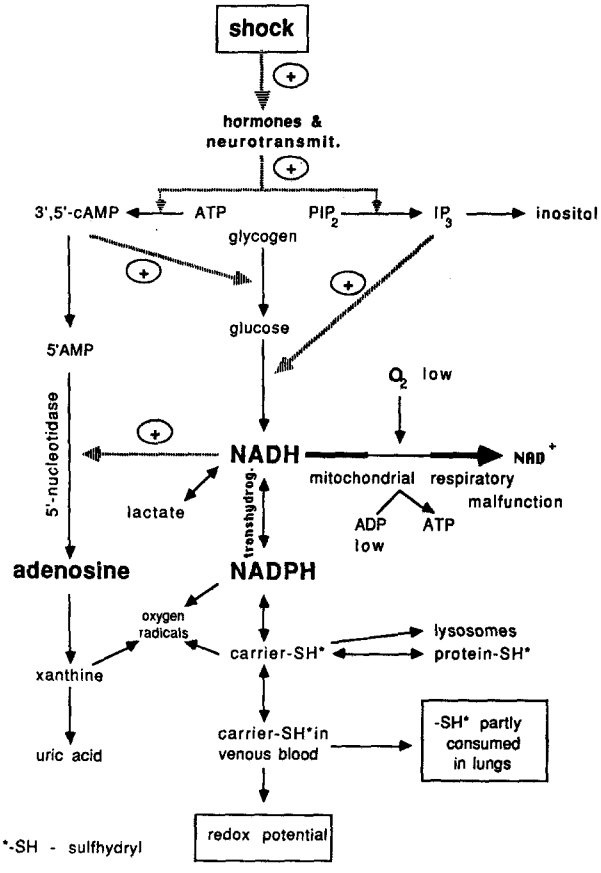

Figure 1. Physiological and biochemical consequences associated with shock. The diagram is an attempt to relate experimental observations to a summary of several known mechanisms of chemical alterations related to shock and the body's physiologic reactions to it.

particularly as related to this study, due to low blood flow, shock, hypotension, and/or hypoxemia. A rational for the effects of hypovolemic shock on the redox state is illustrated in figure 1 and described as follows:

The physiologic reaction to severe and prolonged hypotension resulting from hemorrhage is seen in hormonal and neurotransmitter responses. Responses are mediated, in part, through cyclic AMP production [3] and phospholipase C catalyzed inositol trisphosphate release [4], resulting in greater glycogen and glucose utilization. Hormone activated metabolism may generate NADH beyond the respiratory limits set by declining organ perfusion rates.

Conversion of NADH to NAD^+ may also be restricted by malfunction of the mitochondrial respiratory system associated with shock [5]. Accumulated NADH stimulates 5'-nucleotidase, which is the enyzme responsible for adenosine release from cells [6]. Furthermore, through trans-hydrogenation, NADPH levels increase. Although NADPH does not leave the cell, other carriers of hydrogen, such as the sulfhydryls and ascorbic acid, may cross the cell membrane and appear in the blood where they can be detected by measurements of the redox potential. Paradoxically, saturated hydrogen carriers, which are known to be protective against so-called oxidative stress, may become instrumental in superoxide production in several ways after ischemia and at the time of reperfusion [7–11].

Continued increase in the level of sulfhydryls may also result in long lasting alterations of the tertiary structure of proteins in various tissues and organs. Proteins, such as enzymes and neuronal and hormonal receptors, may therefore have altered function [12, 13]. These alterations could remain in effect even after restoration of blood flow, and, if allowed to persist, may contribute to organ failure [14].

The rate of recovery depends upon resumption of mitochondrial respiration, not only for improved energy production, but resumption of mitochondrial respiration may also be needed to relieve tissues from this hydrogen load. However, mitochondrial respiration usually does not return to normal immediately after shock, in spite of blood and other fluid replacements [15, 16].

In this study, redox measurements were made in the blood of rabbits subjected to shock to see how the potential may change, and how long it would remain altered after reperfusion. We were also interested to see if these changes may lend themselves to modification by a mild oxidizing agent (denatured albumin) to correct any observed changes in redox potential.

Methods

This study was carried out in 15 rabbits weighing 1.6-3.4 kg. Anesthesia was induced with sodium thiamylal (20 mg/kg). Local infiltrative anesthesia with xylocaine was used for all incisions. A tracheostomy was performed and the animal mechanically ventilated. Isoflurane, at a concentration of 1.2 volume % with a 2–2.5 l/min flow of oxygen, was used to maintain anesthesia during the experiment. The right carotid artery was cannulated with a polyethylene catheter for withdrawal and infusion of blood, and the left internal jugular vein cannulated to infuse test solutions. The right femoral artery was cannulated for continuous blood pressure monitoring. Hemorrhagic shock was produced by drawing blood rapidly from the carotid artery cannula into a heparinized syringe to produce a mean arterial blood pressure of 40 mm Hg. This pressure was then maintained for 90 min and the blood was returned. Blood redox potentials and blood pH were measured at 30-min intervals. Arterial pO_2 was also measured. For measurement of oxidation-reduction potential, blood was withdrawn and injected into the redox cell (not calibrated), which consisted of a carbon electrode and a silver/silver chloride reference electrode (fig. 2) [17]. There it was allowed to equilibrate. The values were corrected for pH [18] (-0.06 volts $\times$ pH). The higher the pH, the lower the potential. Blood pH was measured with a radiometer system. After 90 min of shock, one group of 10 animals was given saline for 1 h. In another group of five animals, human serum albumin was given for 1 h, at a rate of 2 g/kg/h.

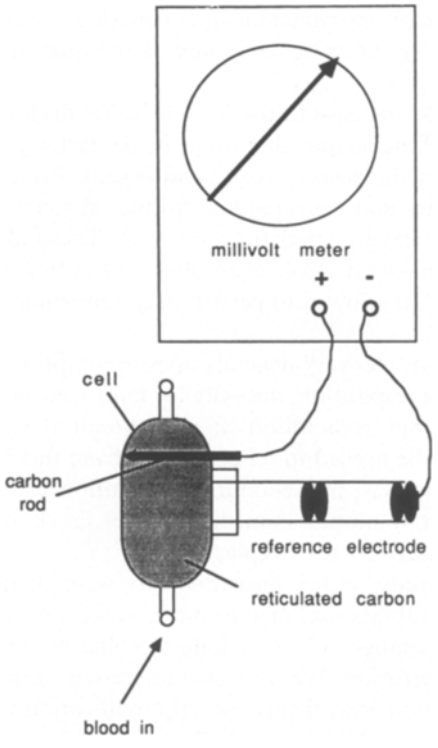

Figure 2. For high surface area, the indicator electrode of the cell was made of unstandardized, reticulated carbon to obtain a stronger electrical signal. The cell was constructed for the exclusion of air. The sample size was 12 ml. The sample was allowed to equilibrate in the cell for 10 min. The same cell was used for all specimens. The blood was reusable after the readings were recorded. The Ag/AgCl reference electrode was checked periodically against a standard Calomel electrode.

Blood redox potential, along with pH, was measured again at 30-min intervals.

Albumin was used as a mild oxidizing agent to determine if it would correct any of the changes in redox potential. The data was analyzed using One Factor ANOVA – Repeated Measure.

Results

The redox potential measurements in the blood of rabbits before and after shock are shown in figure 3. The potential reading corrected for pH showed that the control arterial redox potential was -8.8 ± 1.3 mV. The control venous reading was -18.0 ± 2.0 mV. A 20-mV drop ($p < 0.0001$) was seen in venous blood after 90 min of shock. This drop did not fully return to control ($p < 0.005$) following a 2-h recovery period. The redox potential dropped about 13 mV ($p < 0.005$) in arterial blood, and also did not fully return to normal ($p < 0.02$) after volume replacement. The A-V difference was 80% higher after shock. The pH dropped as expected in arterial and venous blood after the period of shock, and did not return to preshock values with return of blood and with continued observation (fig. 4). The high pH and pO_2 in the initial preshock period was the result of increased ventilation to be certain that a lower redox state and pH did not occur before shock.

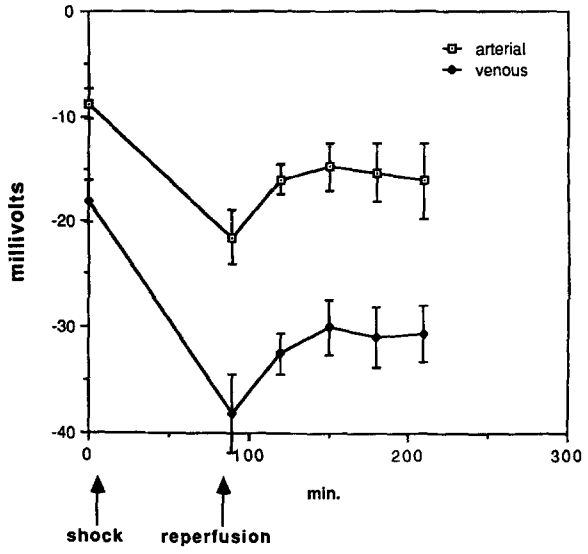

Figure 3. Redox potential in blood of rabbits before and after shock. Compared to control, the p values for the redox potentials of venous blood were $p < 0.0001$ at 90 min, and $p < 0.0005$ for all other points. Compared to control, the p values for the redox potentials of arterial blood were $p < 0.005$ at 90 min and $p < 0.02$ for all other points.

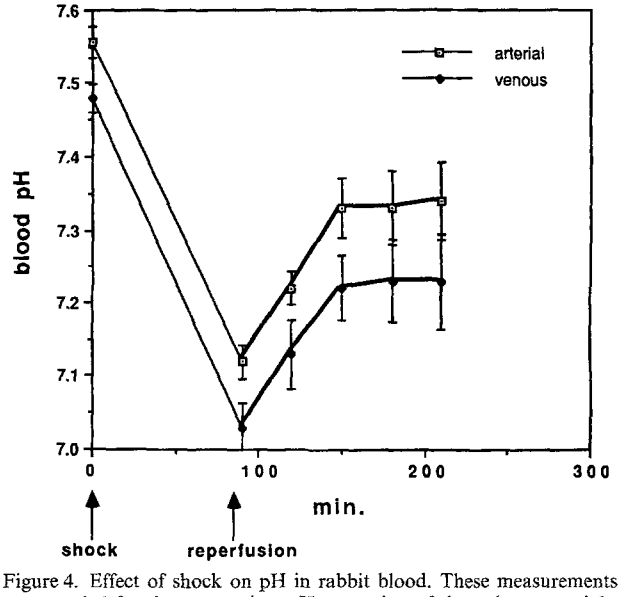

Figure 4. Effect of shock on pH in rabbit blood. These measurements were needed for the appropriate pH correction of the redox potentials. The acidotic state reflects the overall post-shock conditions of the animals. The average arterial pO_2 was 463. This fell to 415 during shock and rose again to the control level in the recovery period.

The effects of albumin infusion on redox potential are shown in figure 5. There was no significant effect of albumin on the redox potential of arterial blood. Albumin infusion corrected the redox potential in venous blood upward to close to control as compared with the control animals; however, this was sustained only during the infusion (fig. 6). After the albumin infusion was stopped, the redox potential in venous blood went back to the previous low value. The A-V difference of redox potential during and after albumin infusion is shown in fig-

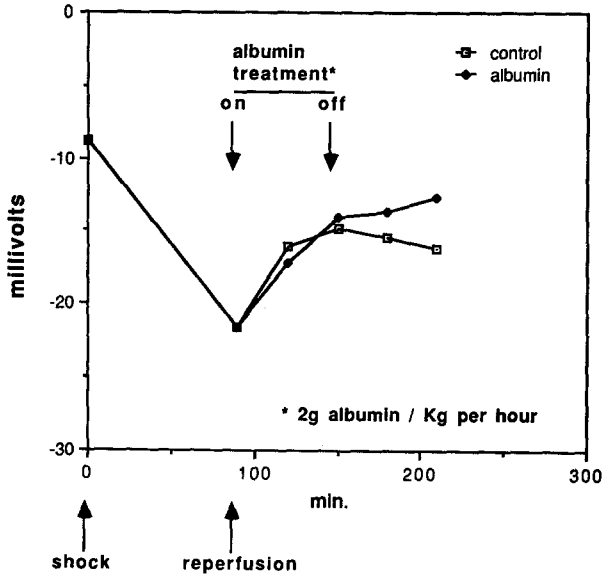

Figure 5. Effect of albumin on redox potential in arterial blood of rabbits after shock. Albumin treatment was without effect on arterial redox potential.

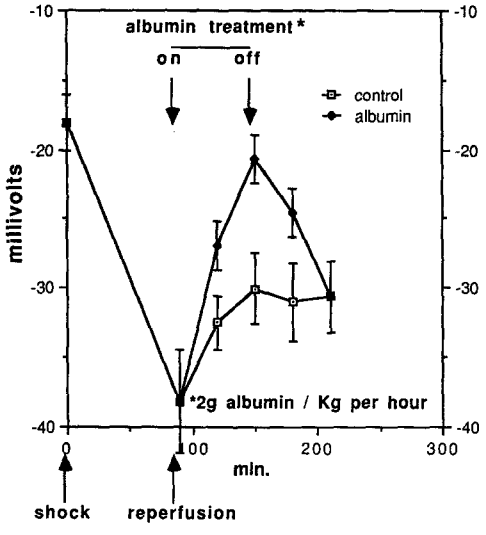

Figure 6. Effect of albumin on redox potential in venous blood of rabbits after shock. After 1 h of treatment with albumin, the venous blood redox potential was higher than the untreated animals ($p = 0.0178$). At this point, the redox potential of the treated animals was the same as the control values.

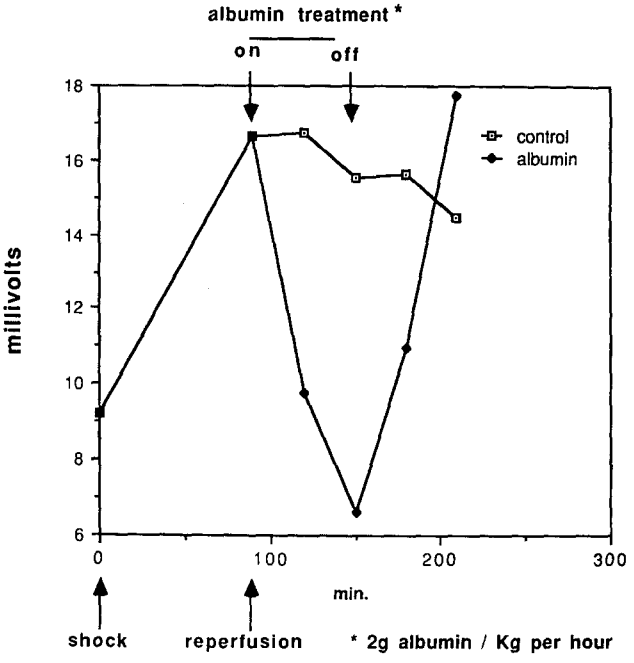

Figure 7. Redox potential A-V difference. The A-V differences demonstrate additional pulmonary consumption of reducing equivalents, and the effect of albumin on this consumption. They also demonstrate the slow decay of the rate of this added pulmonary hydrogen consumption with time.

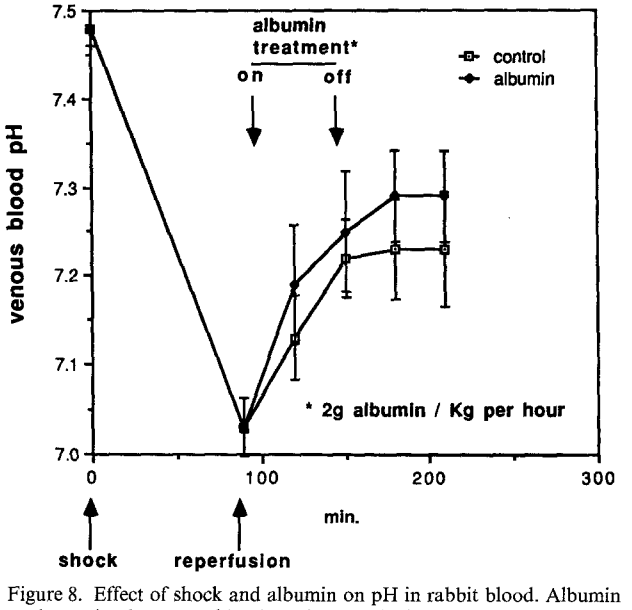

Figure 8. Effect of shock and albumin on pH in rabbit blood. Albumin tends to raise the venous blood pH, but not significantly. The pH changes due to albumin do not resemble the changes observed in the redox potential.

ure 7. The A-V difference returned to the previous level after the infusion was stopped. Redox potential in untreated venous blood did not reach the normal level ($p < 0.005$) over 2 h of observation after shock. There were no significant changes in pH due to albumin (fig. 8).

Discussion

These data show that there is a downward change in redox potential due to shock which is only minimally corrected two hours after restoration of blood volume. The A-V difference almost doubled and reperfusion did not decrease this difference significantly, even though pO_2 returned to control levels. It appeared that hydrogen equivalents coming from muscle and other organ tissues were consumed in the lungs for the most part, but not sufficiently to bring arterial redox potential to control values.

Possibly, there are several pharmacologic ways by which redox potential may be influenced (fig. 9). One way is by

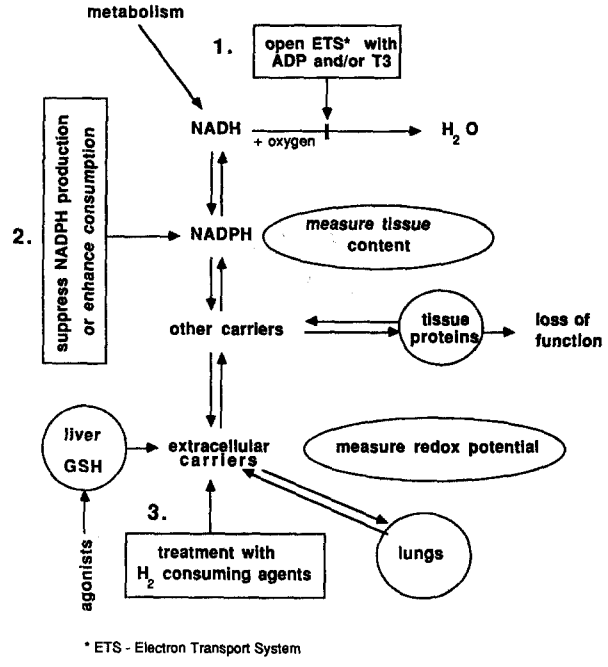

Figure 9. Options for correction of redox potential after shock. Comparison of the intervention with albumin, used in this study, to the other possible means for modifying a reductive load.

improving mitochondrial respiration, perhaps further enhancing mitochondrial hydrogen consumption with agents uncoupling oxidative phosphorylation (fig. 9–1). Another way is to bypass $NADP^+$ reduction in the pentose cycle with the use of ribose or gluconate in place of glucose (fig. 9–2). Another way would be to use substrates such as ammonia, which require NADPH, in its conversion to urea (fig. 9–2).

The method we chose was the infusion of a hydrogen consuming substance to raise the blood redox potential (fig. 9–3). Human albumin was chosen for this because it has many disulfide bridges which, as the result of denaturation, become exposed and accept hydrogen from other carriers to relieve the strain of its own structure[19]. Here, albumin treatment was effective, in that albumin consumed hydrogen as had been predicted. The albumin effect seemed to replace the oxidation which had been observed to take place in the lungs. However, quantitatively, it could not affect a lasting change during 1 h of treatment using 2 g of albumin per kg of rabbit weight, since the redox potentials returned to those of the untreated animals when the treatment was discontinued. We believe that the effect of albumin was specific and not related to any general circulatory effect such as oncotic pressure because the redox potential went down again after the albumin infusion was stopped.

The reductive load imposed on the animal by hypovolemic shock appeared to be rather large and not readily reversed by reperfusion. Extrapolation of the rate of decline of the A-V difference indicates that more than 8 h would have to pass for the lungs to consume these reducing equivalents. This additional pulmonary hydrogen consumption may not be without consequence to the lungs.

The reductive load due to shock may impinge on the recovery of the entire animal if cellular respiration is not adequately restored during that period. We therefore direct attention to the redox condition, confronting a cell where hydrogen levels accumulate beyond a physiologic range. We propose the use of the term 'reductive load' (or 'hydrogen load') for this as opposed to the term 'oxidative stress' which may be associated with the lack of reducing equivalents. A third term, 'oxygen radical challenge', should be added since these species can be derived dependent or independent of oxidative stress or reductive load. Effective redox control may be required to counteract the reductive load and to prevent injury at reperfusion[20]. Such injury has been linked to superoxide formation (oxygen radical challenge in the presence of reductive load) and further loss of energy production[21–23]. Reductive load due to shock may originate in tissue under the influence of stress hormones and resulting ischemia-hypoxemia. Glucose metabolism is accelerated, leading to hydrogen accumulation. This is fueled by increased phosphorylase activity and alpha-1 adrenergic stimulation of glycolysis[24]. Effects of this reductive load may arise from the avalanche of reducing equivalents impacting the system from NADH to NADPH through vast sulfhydryl accumulation. This could destabilize lysosomes[25], which are already endangered by low ATP levels[26]. The release of hydrolytic enzymes may result[27]. Elevated levels of NADPH and sulfhydryls are also suspected of being instrumental in superoxide production during reperfusion. This may take the form of auto-oxidation of sulfhydryls[7,8], NADPH involved redox cycling[9], cytochrome P_{450} activity[10] and increased oxygen burst due to NADPH oxidase in activated polymorphonuclear leukocytes (PMN). Reductive load-induced antigenic proteins of the endothelium may be related to adhesion and activation of PMN, creating reactive oxygen and the proximity for damaging cells[11].

Numerous other proteins, including enzymes and neuronal and hormonal receptors, may also be subjected to alteration and loss of their function, and these altered proteins may be prone to increased rates of proteolysis because of exposure to increased sulfhydryls[28].

NADH levels remain elevated because of low organ respiration which may be due to continued lack of adequate perfusion, or due to limiting low levels of mitochondrial ADP, restricting respiration[29], and/or mRNA needed for replacement of respiratory enzymes[30]. It has been reported that NADH stimulates 5′-nucleotidase[6]. This enzyme is responsible for the release of adenosine from cells, thus decreasing the nucleotide pool needed for the regeneration of ATP after shock. This enzyme may remain active even after restoration of blood volume and perfusion, and this activity may be the continued manifestation of an injurious process initiated in shock and exacerbated by reperfusion. Because of this, and en-

hanced oxygen transformation into free radicals aided by hydrogen, it is possible that part of the reperfusion injury may be perpetuated by this residual reductive load. If this is true, reperfusion injury may be lessened by the removal of excess NADH, NADPH and sulfhydryls.

Because of the apparent size of this pool, this may be best accomplished by improving mitochondrial respiration. Normally, in the cell, the rate of mitochondrial respiration is matched by the metabolic rate for hydrogen formation to prevent hydrogen excess. Thyroid hormone promotes the removal of hydrogen by uncoupling oxidative phosphorylation. Enhanced mitochondrial respiration at reperfusion may benefit the shocked animal by consuming NADH, the positive modifier of 5'-nucleotidase, and other reducing substances that may be stimulating and/or assisting superoxide formation. This could preserve the nucleotide pool and decrease superoxide induced cell damage.

Dogs with low baseline serum levels of thyroid hormones died if subjected to controlled levels of shock while those with higher hormone values survived [31]. Hypothyroidism also abolished the hyperdynamic response to sepsis and increased susceptibility to sepsis [32]. A thyroid-induced increase in hydrogen consumption may be the advantage that the immature individual has in recovering from ischemic episodes [33]. Murphy et al. found that the quantity of adenine nucleotides lost due to global myocardial ischemia was age related [34]. The myocardium of immature animals consistently had more AMP and less inosine than older animals. This suggests that the immature animal had less 5'-nucleotidase activity. At an early age, thyroid function is the highest of the entire life cycle. Perhaps, their higher thyroid function lowered the concentrations NADH, which is a positive modifier of this enzyme.

Further studies may show that organs are affected in many ways by a reductive load, and it is possible that any one of these effects could be decisive in the balance between recovery and death.

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