Kidney International, Vol. 49 (1996), pp. 314–326

EDITORIAL REVIEW

Rhabdomyolysis and myohemoglobinuric acute renal failure

Muscle accounts for approximately 40% of total body mass and falls victim to a wide variety of toxic, ischemic, infectious, inflammatory, and metabolic insults. The final result of these diverse assaults may be muscle fiber dissolution, or rhabdomyolysis, resulting in the release of potentially toxic intracellular components into the systemic circulation. The rhabdomyolysis syndrome has been recognized for centuries. Indeed, the Bible alludes to its occurrence [1]. However, it was not until Bywaters' and Beall's classic description of the "crush syndrome," a result of the bombing raids of London during World War II, that the renal complications of rhabdomyolysis became firmly entrenched in the medical literature [2]. Since then, a plethora of non-traumatic causes of muscle necrosis have been recognized, and today rhabdomyolysis is considered a leading cause of acute renal failure (ARF). For example, at Harborview Medical Center, a Seattle municipal hospital, it accounts for approximately 10 to 15% of all ARF cases. In recent years, a number of important pathophysiologic insights into the nature of rhabdomyolysis-induced ARF have emerged, offering potentially new prophylactic and therapeutic approaches. Therefore, this article has the following goals: (1) review the pathophysiology of myonecrosis, in general, and some of its specific causes; (2) discuss how muscle necrosis can negatively impact the kidney, potentially culminating in tubular necrosis and ARF; and (3) using this pathophysiologic information as a backdrop, review current and possible future pharmacologic approaches for the management of this dramatic and often life threatening "myo-renal syndrome."

Muscle structure, function, and the development of rhabdomyolysis

Muscles are comprised of innumerable elongated multinucleated cells (myocytes), created during embryogenesis by cell fusion. Each cell forms a fiber which is enclosed within endomysial connective tissue. Bundles of adjacent fibers are surrounded by perimysial connective tissue, forming muscle fascicles. The fascicles are separated from each by the epimysium, a dense connective tissue which contains the organ's vascular and neuronal elements. The functional "motor unit" is comprised of the anterior horn cell within the spinal cord, its axon, and the muscle fibers it supplies [3]. While fibers within a given motor unit are scattered throughout the muscle (that is, a "mosaic" pattern of distribution), each of the fibers within a given unit are metabolically similar, being of one of two types. "Slow," or type 1, fibers are characterized by oxidative metabolism, and hence, they have abundant mitochondria and a high myoglobin content. The latter comprises ~ 2% of total muscle weight and functions to store oxygen for mitochondrial consumption during periods of increased oxidative metabolism. In contrast, "fast," or type 2, fibers are predominantly glycolytic; hence, they have relatively few mitochondria and little myoglobin content (that is, "white" as opposed to "red" muscle). Given these distinctly different metabolic profiles, it is not surprising that "slow fibers" are utilized for prolonged, sustained muscular activity (for example, maintenance of posture or continuous athletic activity). Conversely, "fast fibers" undertake sudden, forceful contractions [3–5].

The myocyte plasma membrane, or sarcolemma, maintains a transcellular electrical potential gradient of approximately −90 mV, largely a result of Na,K-ATPase activity. In response to a neuronal impulse, the nerve endings release acetylcholine which opens acetylcholine-gated sarcolemmal sodium channels. Depolarization and an action potential result. As the depolarization wave traverses the muscle fiber, Ca^{2+} is released from the endoplasmic (or sarcoplasmic) reticulum. The released (free) Ca^{2+} reverses troponin-tropomyosin inhibition of actin-myosin binding, ATP is cleaved by myosin-associated ATPase and contraction results. Relaxation is effected as Ca^{2+} is actively transported back into the sarcoplasmic reticulum via an ATP dependent process, permitting re-establishment of the troponin-tropomyosin inhibition of actin-myosin binding. Finally, ATP is required for restitution of the resting sarcolemmal electrical potential via Na,K-ATPase activity. From these brief considerations, it is clear that ATP is critical to muscle contraction, relaxation, and maintenance of baseline myocyte homeostasis [3–5].

As will be discussed below, most forms of muscle necrosis are triggered by derangements in oxidative or glycolytic energy production and the resulting ATP depletion. The latter causes profound disturbances in myocyte ion homeostasis with cytosolic Ca^{2+} overload being a result. The latter is widely purported to be the critical trigger initiating myocyte dissolution [3]. Several potential and overlapping mechanisms lead to myocyte Ca^{2+} overload, some of which are briefly discussed: *First*, direct sarcolemmal damage, such as from physical/exertional trauma or toxic injury, can permit direct Ca^{2+} influx across the sarcoplasmic membrane [3], as dictated by a steep extracellular-intracellular concentration gradient (~10,000:1). *Second*, in the presence of concomitant ATP depletion, a failure of Ca^{2+} ATPase-driven Ca^{2+} efflux results, permitting a sustained increase in myocyte free Ca^{2+} content. *Third*, ATP depletion interferes with normal intracellular Ca^{2+} sequestration within the sarcoplasmic reticulum and the mitochondria. *Fourth*, with progressive mitochondrial and sarcoplasmic damage, Ca^{2+} which is normally stored within these organelles can be released, further contributing to the Ca^{2+} overload state. *Fifth*, Na^+ influx down its transmembrane gradient stimulates Na^+/Ca^{2+} exchange, which further depletes the residual ATP pool, while at the same time contributes to the intracellular Ca^{2+} burden. Thus, a failure of Ca^{2+} sequestration, increased influx, and decreased efflux synergistically produce lethal

Received for publication August 23, 1995
and in revised form October 5, 1995
Accepted for publication October 16, 1995

Table 1. Causes of rhabdomyolysis

Direct muscle injury
Crush syndrome, including pressure (coma-induced) necrosis
Electric shock, freezing, burns
Excessive exercise
Sports/military training
Seizures/myoclonus
Ischemic necrosis
Vascular occlusion/external compression
Metabolic disorders
Diabetic ketoacidosis/non ketotic hyperosmolar coma
Hypothyroidism
Electrolyte disturbances (hypokalemia/hypophosphatemia)
Water intoxication
Sepsis syndrome (bacterial, viral; hyperthermia)
Inflammatory myopathies (polymyositis; infectious myopathies)
Hereditary disorders [44]
Glycogenolytic enzyme deficiencies
(such as McArdle's syndrome)
Defective lipid metabolism
(such as carnitine palmitoyltransferase/carnitine deficiency)
Miscellaneous
(such as malignant hyperthermia/neuroleptic malignant syndrome)
Drugs
for example, alcohol, cocaine, amphetamine, opiates, neuroleptics, clofibrate, lovastatin
Toxins
for example, Tetanus, typhoid, staphyloccal, snake/insect toxins

Ca^{2+} overload. Once a critical free Ca^{2+} concentration develops, neutral proteases (such as calpain), phospholipases (such as PLA_2), and presumably other degradative enzymes are activated, resulting in myofibril and membrane phospholipid damage, respectively [3]. Selected byproducts of these reactions, most notably fatty acids and lysophospholipids, can potentiate the resulting injury by altering ionic transport or via direct membrane lytic effects. Furthermore, Ca^{2+} damaged mitochondria may generate large amounts of superoxide in the ubiquinone region of the respiratory chain; thus, superimposed oxidant stress develops [6]. The net result of these multiple, overlapping, and often self sustaining processes is myocyte lysis with release of toxic intracellular constituents into the extracellular microenvironment. Local accumulation of these products may cause microvasculature damage, producing capillary leak and rising intracompartmental pressures [6, 7]. Neutrophil adherence to damaged endothelial cells can further compromise tissue perfusion [7–13], thereby potentiating tissue ischemia and the evolving muscle damage. With this as a background, some relatively common or illustrative conditions which can trigger this injury cascade will be presented. A more exhaustive list of potential causes of rhabdomyolysis appears in Table 1.

Muscle injuries which can culminate in rhabdomyolysis

Crush syndrome

Crush injuries sustained during the Battle of Britain formed the basis for Bywaters' and Beall's classic descriptions of rhabdomyolysis-associated ARF [2]. While this form of injury is now seen most often as a sporadic event, it can still occur in "epidemic" proportions, as tragically demonstrated by U.S. Marines bombing of Beirut barracks in 1983 [14], and by the Armenian earthquake of 1988 in which 600 patients developed myoglobinuric ARF [15]. The pathophysiology of crush syndrome-induced muscle injury, while seemingly straightforward, is actually quite complex. The earliest insult may be "pressure-stress myopathy" in which external pressure/tension on muscle causes increased sarcolemmal Na^+ and Ca^{2+} influx down their concentration gradients [16–18]. External pressure-induced occlusion of the microcirculation also occurs, rapidly depleting ambient and myoglobin oxygen content. While some high energy phosphate reserves exist in the form of creatine phosphate, these stores are soon depleted, glycogen stores are exhausted, and severe ATP depletion ensues. Despite this onerous situation, cell viability may remain intact for considerable periods of time, for a number of reasons [19]: (1) pressure-induced vascular occlusion limits Ca^{2+} delivery to ischemic tissues; hence, this may stave off critical muscle Ca^{2+} loading, forestalling the onset of cell necrosis. (2) Mitochondrial oxygen free radical production is markedly reduced during ischemia because oxygen deprivation stops electron transport; thus, decreased rather than increased mitochondrial H_2O_2 and superoxide production may result [20]. (3) Intracellular/extracellular acidosis, a correlate of tissue ischemia, has a remarkably potent, and apparently generalized cytoprotective effect [21–24]. With restitution of blood flow, each of these intrinsic homeostatic mechanisms are reversed: Ca^{2+} influx occurs, brisk mitochondrial oxygen free radical formation may result, and tissue acidosis recedes. Furthermore, restoration of blood flow allows for neutrophil influx, which can re-occlude the microcirculation and release proteases and free radicals into the microenvironment. Hence, a new wave of injury results [19]. These considerations indicate that although the crush lesion clearly *sets the stage* for rhabdomyolysis, many of its critical components are *enacted* during the reperfusion period. It is also during the latter time that myoglobin gains ready access to the circulation, and extracellular fluid depletion results because of muscle/soft tissue fluid sequestration. These two factors are critical to the evolution of crush syndrome-induced ARF, as will be discussed at length later in this review.

Exertional rhabdomyolysis

Strenuous physical exercise is probably the most common cause of severe rhabdomyolysis, particularly when it is performed by untrained individuals in hot, humid weather. Therefore, it is not surprising that outbreaks of myonecrosis have been noted at Southern military bases and summer sports training camps [25, 26]. However, non-athletic exertion can also produce this result, as evidenced by the onset of myoglobinuria following generalized convulsions or septic rigors [27]. The specific events leading to exertional myonecrosis have only been partially clarified, and the syndrome develops in stages [28]: The *initiating stage* is believed to result from mechanical muscle fiber injury, induced, in particular, by eccentric contractions and when the muscle is in an elongated condition (such as down hill walking [28–30]). These factors produce excessive tensile stress and heat generation, resulting in muscle fiber injury. Continuous contractions can also compromise muscle capillary blood flow. When coupled with progressive glycogen and creatine high energy phosphate consumption, ATP depletion results. The *Ca^{2+} overload stage* is thought to be initiated by a Ca^{2+} influx across a mechanically damaged myocyte sarcolemmal membrane [28–31]. For reasons noted above, concomitant energy depletion either initiates or markedly contributes to this result. Ca^{2+} overload is then believed to initiate the

autogenous phase, during which phospholipase and protease activation, mitochondrial dysfunction, and free radical formation perpetrate sarcalemmal injury, culminating in cell death [28, 32]. Muscle hyperthermia (as much as 43°C), induced by strenuous exercise in concert with impaired heat dissipation (by hot humid conditions), undoubtedly plays a major role in these processes [33], for at least two reasons. First, a rising muscle temperature markedly increases metabolic rate, and hence, ATP consumption. While this may be well tolerated by otherwise normal tissues, it can cause profound ATP depletion in cells with already compromised glycolytic or mitochondrial energy production [34]. Second, enzymatic degradative reactions are highly thermal dependent, generally increasing by ~10% for each 1°C increment. This not only accelerates tissue injury, but it can also convert sublethal into lethal cell damage [35].

Of considerable interest is the mechanism by which prior muscle conditioning prevents the above sequence of events [26, 33]. It presumably reflects a cellular adaptation to prior exercise-induced sublethal injury. The exact pathways by which it is effected remain to be defined. However, it is noteworthy that sublethal injury-induced "cellular conditioning" may not necessarily be confined to skeletal muscle. For example, the post-ischemic myocardium can acutely develop partial resistance to further ischemic injury (the "stunning phenomenon" [36]), and during the early recovery phase of ARF, tubules demonstrate heightened resistance to further toxic or ischemic insults [37–40]. Whether these phenomena reflect common adaptive mechanisms remains unknown.

Intrinsic myopathies

The first report of a presumptive metabolic disorder causing recurrent myonecrosis appeared in 1884 [41]. Approximately 30 minutes after the onset of strenuous exercise, horses were noted to develop muscle tremors, weakness, and limpness, followed by myoglobinuria. In 1910, a similar disorder was noted in a 13-year-old boy [42]. However, it was not until the detailed reports of McArdle in 1951 that the study of hereditary disorders which trigger rhabdomyolysis began in earnest [43]. Since that time, a panoply of muscle energy production disturbances have been documented, of which McArdle's disease is a prototype. Excellent reviews of this area are available [3, 44], and a detailed description of this subject is beyond the scope of this review. However, two general issues are worth noting. First, these disorders typically present as recurrent episodes of muscle pain with or without overt myoglobinuria. Clues to an underlying metabolic disorder include: (a) onset in childhood; (b) a positive family history; (c) mild baseline creatine phosphokinase elevations; (d) onset of clinically overt muscle necrosis with either mild exercise or no obvious precipitating event; and (e) recurrent attacks. Second, divergent defects can give rise to this pathology, including deranged glycolysis, altered mitochondrial function, and impaired muscle excitation.

McArdle's disease. McArdle's disease is the most common cause of the first set of disorders and is believed to be due to myophosphorylase deficiency. Since this enzyme promotes glycogenolysis, its deficiency presumably causes defective anaerobic energy production in type II fibers, culminating in exercise-induced ATP depletion and myonecrosis.

Carnitine palmitoyl transferase deficiency. This is the prototypic mitochondrial disorder giving rise to recurrent rhabdomyolysis. This enzyme is required for transport of long chain fatty acids across the inner mitochondrial membrane; hence, its deficiency leads to defective mitochondrial energetics, and presumably exercise-induced ATP depletion.

Malignant hyperthermia. The third general category of genetic muscle disorders leading to rhabdomyolysis is represented by malignant hyperthermia. Classically, it presents with the onset of muscle rigidity and contractures, a rapidly rising body temperature (as much as 1°C every 5 min; reaching $\geq$ 43°C), and myonecrosis. Although the exact genetic and metabolic basis for this disorder remain unknown, it is believed to be due to altered excitation-contraction coupling, resulting in increased sarcolemmal Ca^{2+} transport, contractures, and increased muscle heat production. That attacks are often (but not invariably) triggered by halogenated hydrocarbon anesthetic and succinylcholine exposure are clues to the diagnosis. Specific diagnostic and therapeutic approaches to these hereditary disorders have recently been reviewed by Brumback, Feeback and Leech [3].

Alcoholism, hypophosphatemia, and hypokalemia

Alcoholism is a leading cause of rhabdomyolysis [27] and may contribute to muscle injury in a variety of ways. For example, it can predispose to or cause trauma, seizures, or coma-induced ("found down") ischemic pressure necrosis. It is also a direct muscle toxin, inducing either chronic or acute myopathy. Knochel has performed much of the pioneering work in this area and readers are referred to several of his excellent reviews of this subject [33, 45–47]. In brief, alcohol intake, independent of concomitant malnutrition, induces substantial changes in muscle ion homeostasis, including increments in Na^+, Cl^-, and Ca^{2+}, and decrements in Mg^{2+}, K^+, and phosphate content. These changes are associated with increased Na,K-ATPase activity, suggesting that ethanol directly injures the sarcolemma, allowing for increased Na^+ entry [33, 48]. A secondary increase in Ca^{2+} may then result via enhanced Na^+/Ca^{2+} exchange. Given calcium's critical role in mediating rhabdomyolysis, these processes may either initiate myonecrosis, or predispose the muscle to concomitant insults, such as electrolyte disturbances, as discussed below.

Hypophosphatemia is a frequent co-existent risk factor for alcohol-induced rhabdomyolysis [33, 45, 46], although the direct mechanisms by which it does so have remained somewhat elusive. A leading possibility is that intracellular inorganic phosphate deficiency limits ATP production, potentially imposing an energy deficient state. The latter would then predispose to intracellular Ca^{2+} loading, ultimately triggering myocyte death. While chronic phosphate depletion can induce myopathy [49], it rarely produces overt rhabdomyolysis in the absence of alcohol intake. Hence, it is postulated that phosphate depletion only initiates myonecrosis in the presence of pre-existent muscle damage [33, 50]. This is particularly likely to occur during a period of caloric, but phosphate deficient, refeeding (such as, glucose infusion). A possible reason for this is that cellular glucose uptake obligates phosphate utilization (glucose 6 phosphate formation), potentially triggering severe intracellular inorganic phosphate- and ATP-depletion. With the onset of muscle necrosis, intracellular phosphate is released. This may rapidly correct the hypophosphatemia, thereby obscuring its original pathogenetic contribution to the muscle injury.

Hypokalemia is the second electrolyte disturbance which may

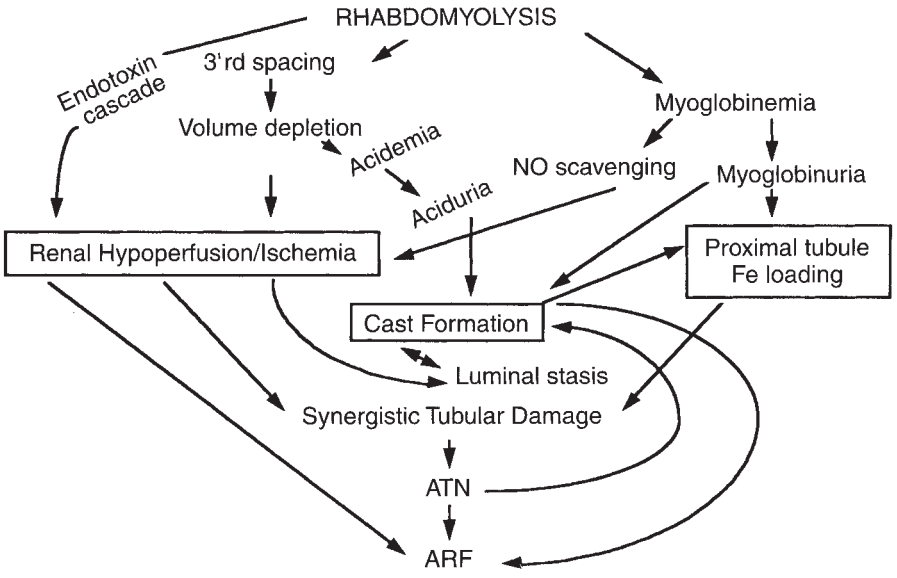

Fig. 1. *Potential pathways underlying rhabdomyolysis-induced renal injury.* Renal hypoperfusion/ischemia, iron mediated proximal tubule cytotoxicity, and cast formation are postulated to play central, but interrelated, mechanisms which lead to proximal tubular necrosis and ARF. Filtration failure ultimately results from some combination of proximal tubular necrosis, tubular obstruction, and a renal vasoconstricted state.

initiate or contribute to rhabdomyolysis-induced ARF [33]. Common underlying causes include physical exercise in hot weather (sweat and urinary K^+ losses), long-acting diuretic therapy, and alcoholism. As with acute hypophosphatemic myopathy, it may be difficult to document hypokalemia with the onset of myonecrosis because of the resulting cellular K^+ loss. Two mechanisms for hypokalemic rhabdomyolysis have been suggested, both of which relate to altered myocyte energetics. First, K^+ depletion can interfere with glycogen synthesis [51]. Second, the exercising muscle may fail to develop reactive hyperemia, a prerequisite for increased oxygen delivery during a period of increased oxygen demand [52]. Since this vasodilatory response is dependent on myocyte K^+ release and the attainment of high muscle interstitial K^+ concentrations (~10 to 15 mEq/liter), a pre-existent K^+ deficiency may prevent this critical adaptive response. Thus, a combination of diminished glycogen reserves and impaired oxygen delivery/aerobic energy production may induce energy depletion which then initiates myocyte death.

Mechanisms of myohemoglobinuric renal injury

The association of rhabdomyolysis with ARF was well established at the time of World War I. However, that myoglobin was directly involved in the induction of the renal damage was not definitively established until Bywaters and Stead induced ARF by myoglobin infusion [53, 54]. Those experimental studies also revealed the two critical factors which predispose to myohemoglobinuric ARF: hypovolemia/dehydration and aciduria. Indeed, it has been repeatedly demonstrated that in their absence, heme proteins have minimal nephrotoxic effects. At the nephronal level, three basic mechanisms underlie heme protein toxicity: *renal vasoconstriction, intraluminal cast formation, and direct heme protein-induced cytotoxicity* [55]. The following discussion will address each of these factors and stress the nature of their critical interactions, as depicted in Figure 1. In this discussion, the renal effects of hemoglobin and myoglobin are considered together (that is, "heme proteins") since they are thought to have the same renal impact.

Renal vasoconstriction

The two most widely used experimental models of heme protein-induced ARF are either heme protein (hemoglobin/myoglobin) infusion into dehydrated/volume depleted animals or intramuscular hypertonic glycerol injection. The latter causes myolysis, hemolysis, and intravascular volume depletion. The importance of the latter and its associated renal vasoconstriction are underscored by the fact that either volume expansion or early renal vasodilator therapy can prevent, or profoundly attenuate, heme protein-induced ARF [56]. That intravascular volume depletion is a near constant correlate of clinical rhabdomyolysis [14, 16, 19], and that hemoglobinuria rarely induces ARF in volume expanded individuals [57], indicate the relevance of these experimental observations.

Several potential mechanisms may contribute to renal vasoconstriction/hypo-perfusion in the setting of rhabdomyolysis: First, muscle necrosis causes dramatic fluid third spacing, leading to intravascular volume depletion. Better indicated that following severe crush injuries, as much as 18 liters of fluid may extravascate into damaged limbs [14]. That aggressive volume repletion (~1 liter/hr) during the early post-injury period can dramatically decrease the incidence of clinical rhabdomyolysis-induced ARF [14, 58, 59] underscores the pathogenetic importance of this volume depleted/renal vasoconstricted state. Second, severe muscle injury can, for unceretain reasons, activate the endotoxin cytokine cascade. Its pathogenetic role in rhabdomyolysis-induced ARF has been convincingly demonstrated by several studies by demonstrating that either active or passive endotoxin tolerance prevents heme protein-induced renal damage [60–62]. Since endotoxemia elicits renal vasoconstriction [63, 64] and can depress left ventricular function [65], it is quite plausible that endotoxin's adverse effects are mediated by an increase in rhabdomyolysis-associated renal vasoconstriction. That endotoxin tolerance abrogates renal vasoconstriction and filtration failure in a sepsis/gentamicin model of ARF lends support to this possibility

[64]. Third, heme proteins scavenge nitric oxide (NO), an important endogenous vasodilator [66–68]. Studies by Brezis et al have demonstrated NO's importance in maintaining renal medullary oxygenation, an area with a tenuous blood supply [69]. That interventions which increase and decrease NO protect against and exacerbate various forms of experimental ARF, respectively, underscore the potential importance of NO in the expression or toxic and ischemic tubular damage [70–76]. Finally, Wright, Rees and Moncada have indicated that NO synthase inhibition in experimental septic shock markedly increases vasoconstriction, producing worse end organ tissue damage and rapid death [77]. Hence, it seems clear that heme protein-induced NO scavenging could directly contribute to renal hypoperfusion and tissue injury in the setting of rhabdomyolysis. Recent demonstrations that NO synthase inhibition worsens, and NO supplementation protects against the glycerol ARF model, respectively, directly support this view [78].

A recent elegant study by Vetterlein et al has provided compelling evidence that myoglobinemia exacerbates renal hypoperfusion in the setting of acute volume depletion [79]. These workers infused myoglobin into normal rats or rats which had been subjected to acute hemorrhagic shock (50 mm Hg). Total renal blood flow was measured by electromagnetic flow probe, and cortical and medullary microperfusion were assessed by a fluorescent dye technique. Whereas myoglobin infusion had no discernible effect on renal perfusion in normal animals, it profoundly exacerbated cortical and medullary hypoperfusion in the hypotensive animals. The potential impact of this change on tissue energetics and cell injury had previously been addressed by a study from our laboratory [80]. Employing a similar protocol to that used by Vetterlein, we noted that myoglobin infusion caused profound reductions in renal cortical ATP concentrations in hypotensive rats. This resulted in a marked exacerbation of tissue injury, resulting in anuric ARF. In data gathered during, but not reported in that study, myoglobin infusion also exacerbated shock-induced hepatic ATP depletion. Clearly, then heme proteinemia can have a profoundly negative impact on cellular energetics and shock-induced tissue injury. It is noteworthy that these adverse effects may be clinically masked by heme's ability to maintain mean arterial pressure (MAP) during acute volume depletion states [80–82], presumably by NO scavenging. This suggests that although heme proteins may help to maintain MAP via an increase in peripheral vascular resistance, ischemic tissue injury may still result.

The above studies strongly suggest that renal vasoconstriction participates in the expression of heme protein-associated ARF via the induction of renal tubular ATP depletion (discussed in greater detail below). However, renal vasoconstriction can also facilitate heme toxicity by increasing cast formation and by promoting proximal tubular cell heme uptake. This occurs via a number of pathways: first, a decrease in GFR prolongs the circulating half life of heme proteins. Since heme endocytosis is a saturable process, most of the filtered load is rapidly excreted. However, if the period of heme proteinemia is prolonged due to a decrease in GFR, more of the total filtered load can undergo reabsorption, thereby exacerbating the extent of proximal tubular heme loading, and hence, cell damage. Second, volume depletion, and hence increased Na and fluid reabsorption, increase intraluminal myoglobin concentrations, favoring cast formation and tubular obstruction; and third, tubular obstruction causes profound luminal stasis, allowing more time for proximal tubular heme reabsorption, as discussed below [83]. These considerations merely underscore that renal vasoconstriction can adversely impact the expression of myohemoglobinuric injury via divergent, but interrelated pathways (Fig. 1).

Heme protein cast formation

The presence of large numbers of distal nephron heme pigment casts have been noted in virtually every study of myohemoglobinuric ARF (see Fig. 2). Since some studies have also revealed associated tubular dilatation [84–86], it has been widely hypothesized that these casts induce tubular obstruction, thereby contributing to the pathogenesis of the renal failure. Despite this assumption, consistent micropuncture data to support this view have been lacking. For example, Oken, Arce and Wilson reported low, rather than high intratubular pressures in rat kidneys four hours following glycerol-induced myohemoglobinuria [87]. The tubules appeared collapsed, rather than dilated, and the casts could be readily expelled by intraluminal fluid infusion under normal pressures. These observations led these workers to conclude that the casts were a *reflection* of the filtration failure (that is, no washout), rather than a *cause* of it. They also could not demonstrate substantial tubular backleak, and hence it was concluded that the ARF had a predominant hemodynamic basis. In a study by Riuz-Guinazu, Coelho and Paz, assessments of tubular pressures were made from 0 to 96 hours following the induction of heme protein ARF, created by methemoglobin infusion [88]. Two populations of tubules were noted. Some were dilated and had normal–to–high intraluminal pressures. This population was predominantly (but not exclusively) found during the first few hours of injury. The second nephron population had collapsed tubules, and was typically observed during the maintenance phase of the ARF. Very similar results to these were obtained by Jaenike, who also used a methemoglobin infusion ARF model [89]. On the basis of these studies, it has been suggested that intratubular obstruction may not be a critical factor underlying heme protein-induced filtration failure. However, it should be noted that concomitant reductions in cardiac output and attendant renal vasoconstriction would be expected to lower glomerular capillary pressure. Hence, even "normal" intratubular pressures could be abnormally high in this setting and therefore, contributed to a decreased GFR. Furthermore, no compelling alternative explanation for persisting filtration failure beyond the period of renal vasoconstriction exists. Thus, the nephronal determinants of the ARF, in general, and the precise role of cast formation, in particular, remain to be defined.

Although one typically views cast formation as being important to the pathogenesis of ARF because of its direct effects on glomerular filtration, we have previously suggested that in the case of heme protein nephrotoxicity, it may also play a critical role in the induction of tubular necrosis. As alluded to above, we have noted that the degree of proximal tubular heme uptake is dramatically increased in the presence of even short-lived urinary tract obstruction. For example, if heme proteins are infused into rats with acute ureteral ligation, giant heme laden endolysosomes form in proximal tubular segments [83]. Similarly, if cast formation is facilitated by infusing heme proteins into aciduric rats (see below), proximal tubular heme endocytosis is, once again, greatly enhanced [83]. Undoubtedly, this reflects the fact that an obstructing lesion induces luminal stasis, thereby providing an extended

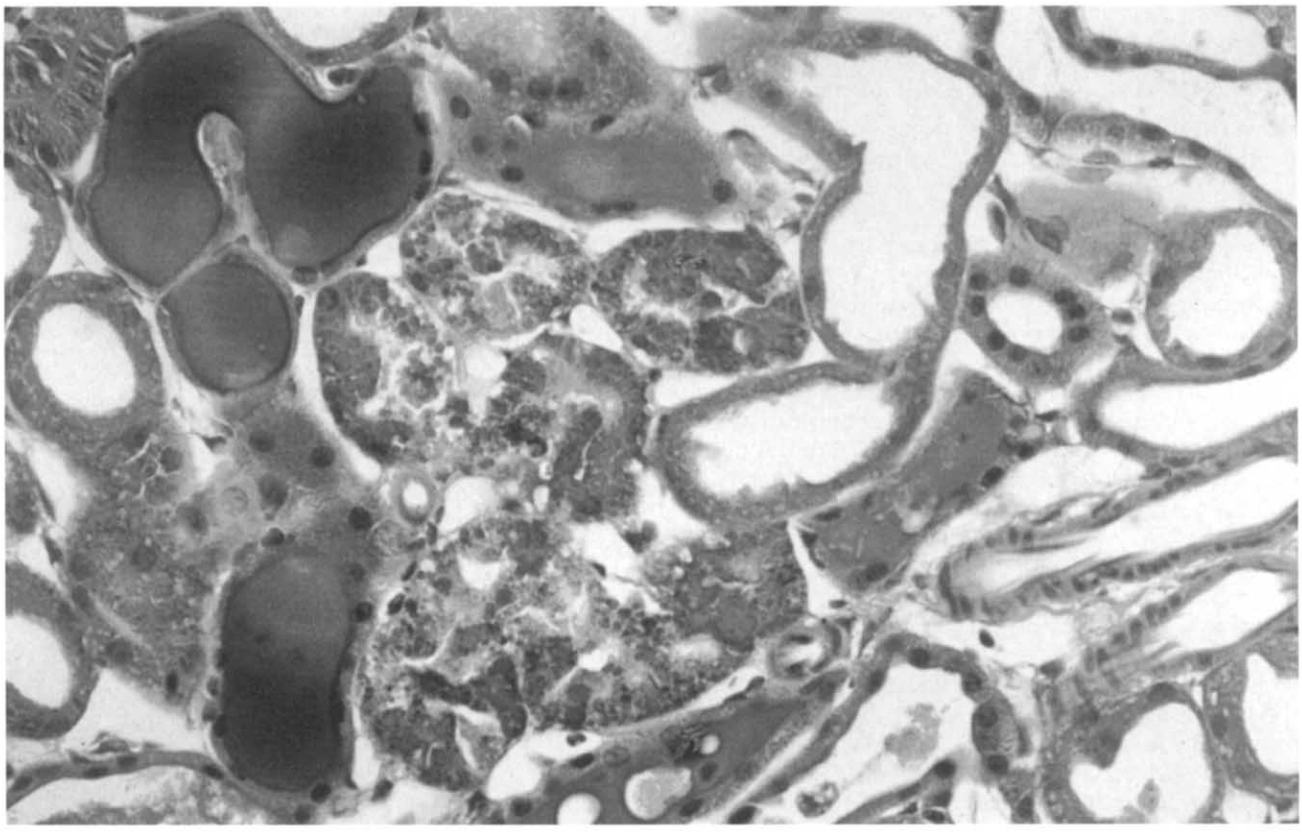

Fig. 2. *Characteristic morphologic changes of heme protein nephrotoxicity (rat kidney 8 hr following heme protein infusion [83].* Distal nephron heme casts are seen (left), and giant heme-filled endolysosomes are observed in occasional proximal tubular cells (middle). Many of the proximal tubular cells show early signs of necrosis, leading to detachment from the basement membrane. Modest tubular dilatation is also observed (right center), suggesting that a component of tubular obstruction exists. (Hematoxylin and eosin stain; perfusion fixed tissue).

period for proximal tubule endocytic transport. Since heme protein cytotoxicity is clearly dose dependent, even transient cast formation could have a profound impact on the evolution of ARF by favoring toxin uptake and, therefore, the development of tubular necrosis. As demonstrated by the above described micropuncture experiments, heme cast-induced tubular obstruction appears to be more prominent during the initiation phase, rather than the maintenance phase, of myohemoglobinuric ARF. Since this early period corresponds with the stage of heme filtration and thus heme endocytosis, even transient tubular obstruction at this time could have a pronounced and prolonged impact on the severity of the ARF.

The mechanism by which heme protein cast formation occurs has been rather well characterized and is largely determined by two factors: (1) the heme protein concentration in the distal nephron; and (2) the characteristics of the urine in which it resides. Since the degree to which heme proteins precipitate from solution is, in part, concentration dependent, the amount of the filtered load (determined in large part by the extent of muscle injury), and the more that load is concentrated along the nephron, the more likely it is that cast formation will result. Since volume depletion and renal vasoconstriction enhance filtrate reabsorption, they can increase intraluminal heme concentrations, facilitating cast development. The second critical factor is urinary pH. For example, if myoglobin is added to normal human urine at a pH of 8.5, a solubility of $\geq$ 50 mg/ml can be achieved (exceeding that which is typically found during experimental myohemoglobinuria [90]). However, as urinary pH is progressively lowered to 5.0 by HCl addition, a progressive loss of myoglobin solubility results, with 98% precipitating by the end of the titration [90]. An *in vivo* correlate of this observation is that renal myoglobin retention progressively increases with progressive reductions in urine pH. For example, we previously observed that when urinary pH was maintained at 8.0, 78% of an exogenous myoglobin load was excreted. Conversely, under aciduric conditions, only 32% of the myoglobin was eliminated [90]. This relationship undoubtedly explains the long-standing experimental observation that aciduria is an important determinant of heme protein-induced ARF. What is less well appreciated, however, is that this pH effect is largely Tamm Horsfall protein (THP) dependent. For example, if myoglobin is added to 0.45% NaCl instead of urine, only 10%, not 98%, precipitates at a 5.0 pH [90]. A characteristic of THP is its propensity to aggregate with other proteins under aciduric conditions, forming casts [91, 92]. Undoubtedly, this is the principal reason why urine facilitates heme protein precipitation under conditions of a reduced pH. That THP is synthesized in the distal nephron [93, 94], and that this is the predominant site at which heme casts reside, further points to its central role to intraluminal heme trapping. Ultimately, cast formation results not just from heme proteins, but also from the evolving heme-induced proximal

tubular necrosis. The underlying mechanisms for the latter are discussed below.

Mechanisms of heme mediated proximal tubular cytotoxicity

In addition to cast formation, the second typical morphologic feature of heme protein-induced ARF is proximal tubular necrosis (Fig. 2). That this lesion ultimately contributes to filtration failure is indirectly supported by the fact that mitigating its expression via pharmacologic interventions attenuates the severity of ARF. Once proximal tubular injury is initiated, a host of cellular degradative reactions and maladaptive responses (for example, loss of membrane polarity, altered cell-cell interactions) are undoubtedly called into play and ultimately contribute to lethal cell injury. The nature of these interactive injury pathways has been the subject of several recent excellent reviews [94–98]. Therefore, the present discussion will stress two factors which are currently believed to play critical roles in the initiation of this injury cascade: ischemic damage and oxidant tissue stress.

Ischemic tubular injury and ATP depletion

It has been recognized for at least 50 years that heme protein nephrotoxicity and ischemic renal damage are inextricably linked [99–101]. As previously detailed, this is probably mediated in part by the ability of heme proteins to intensify renal vasoconstriction, thereby converting an episode of renal hypoperfusion into a severe ischemic insult [79]. Therefore, it may well be that myohemoglobinuric ARF is, in large part, ischemia induced. This possibility is further supported by work from Trifillis, Kahng and Trump in which substantial decrements in renal cortical ATP content were noted early in the course of glycerol-induced ARF [102].

However, it is also highly likely that an adverse heme protein-ischemic interaction exists directly at the proximal tubular cell level (that is, independent of hemodynamic influences). For example, if an ordinarily non toxic dose of hemoglobin or myoglobin is infused into a rat prior to the induction of a fixed ischemic renal insult (renal artery occlusion; RAO), a marked potentiation of ischemic tubular necrosis and filtration failure result [99–101, 103]. In one of these studies, no detectable change in renal perfusion was noted either before or after RAO [103], strongly suggesting that the ischemic damage was potentiated *directly at the proximal tubular cell level.* This conclusion was further supported by the fact that this adverse heme effect was expressed without any change in renal cortical/outer medullary ATP content [103]. Hence, heme infusion seemingly worsened the *expression* of the ischemic damage, rather than the *severity* of the ischemic insult *per se*.

Since it has long been assumed that porphyrin iron is the specific mediator of heme protein cytotoxicity [104], we evaluated whether this was the specific determinant of the above "ischemia-potentiating" effect. If so, this would provide specific clues into potential mechanisms. Towards this end, rats were infused with 25 mg of either myoglobin or non-iron-containing low molecular weight proteins (anionic or cationic lysozyme, ribonuclease) [103]. Each rat was then subjected to a 25-minute ischemic insult (renal artery occlusion). Surprisingly, each of these readily filtered low molecular weight proteins markedly and equally potentiated the severity of the superimposed ischemic insult. This was despite the fact that none of the proteins in the dose employed exerted an independent toxic effect. In contrast, albumin infusion (which undergoes minimal filtration) had no discernible impact. Next, it was demonstrated that cytochrome C, a readily filtered but relatively poorly reabsorbed heme protein, did not exacerbate superimposed ischemic damage. Thus, these experiments indicated that endocytic protein reabsorption (or some secondary consequence of it), and not cell iron loading *per se*, is in large part responsible for heme's ability to potentiate ischemic damage. The low molecular weight protein infusions caused an early increase in ischemia-triggered brush border membrane blebbing [103]. We speculated that the protein endocytic reabsorptive process, or the resulting plasma membrane lipid trafficking/"remodeling" [105], may directly sensitize the plasma membrane to ischemia-triggered attack [103].

To further explore this possibility, we have recently conducted a series of experiments using a newly devised *in vitro* model of heme protein cytotoxicity [106]. To induce a clinically relevant form of proximal tubular heme protein exposure, rats or mice were injected with glycerol. After allowing a two to four hour period for heme protein endocytic reabsorption, the kidneys were removed and proximal tubular segments (PTS) were isolated. During subsequent *in vitro* incubations, these tubules typically express progressive heme-induced cytotoxicity [106]. Since phospholipase A_2 is probably the critical determinant of ischemia-triggered lethal membrane injury [95, 96], we then used this *in vitro* model to assess whether heme endocytic transport/membrane remodeling does, in fact, increase the plasma membrane's susceptibility to PLA_2 attack. To this end, control and heme loaded PTS were incubated with exogenous PLA_2 [107]. Over the next hour, lethal cell injury and PLA_2-induced deacylation were assessed by lactate dehydrogenase release and arachidonic acid release, respectively. Whereas PLA_2 addition to normal PTS induced no cell death and minimal deacylation, synergistic damage occurred in the heme-loaded PTS, as assessed by both injury parameters [107; unpublished data]. These data strongly supported the hypothesis that heme endocytosis directly sensitizes the plasma membrane to PLA_2 attack. In contrast, when PLA_2 was added to PTS which were damaged by hypoxia, a decrease rather than an increase in cell death resulted [107, 108]. Thus, PLA_2's ability to exacerbate injury in the heme loaded tubules was not merely due to a non-specific 'injury potentiating' effect. The underlying mechanisms for these phenomena remain subjects of ongoing investigation.

Another pathway by which heme proteins can contribute to ischemic tubular damage is via an apparent adverse effect on cellular energetics, independent of their negative impact on renal perfusion. The following experiment provides support for this concept. When normal rats were subjected to an intravenous myoglobin infusion, an approximate 25% reduction in renal cortical ATP concentrations developed without any discernible change in renal hemodynamics [80]. This result could not be reproduced by infusing a non-iron containing low molecular weight protein (ribonuclease), suggesting an iron dependent reaction. This conclusion was further supported by the fact that when myoglobin was infused in the presence of an iron chelator (deferoxamine; or DFO), no diminution in ATP resulted. In summary, these considerations underscore that multiple and potentially overlapping mechanisms for the heme protein-induced exacerbation of ischemic renal injury exist: (*i*) heme proteins can intensify renal vasoconstriction in the setting of volume depletion;

(*ii*) they can adversely affect ATP availability via a non hemodynamic, iron dependent mechanism; and (*iii*) heme protein endocytic reabsorption can directly sensitize tubular cells to the adverse consequences of superimposed ischemia-triggered injury pathways (such as PLA_2 attack). Given these multiple adverse interactions, it is not surprising that renal ischemia and heme protein nephrotoxicity have been inextricably linked in the literature.

Heme iron-induced oxidant stress

In 1969, Bunn and Jandl injected haptoglobin deficient animals with tracer amounts of ^{59}Fe labeled hemoglobin to determine its metabolic handling by the kidney. They noted that the hemoglobin rapidly dissociated within the circulation into $\alpha\beta$ dimers (thereby halving its size, permitting rapid filtration) and this was followed by proximal tubule heme endocytic reabsorption [109]. Once within proximal tubular cells, the porphyrin ring was rapidly catabolized, releasing its iron content. The latter was then transferred to ferritin. Subsequently, the intratubular ^{59}Fe disappeared over several weeks. In contrast, if large amounts of hemoglobin were administered, the tubular reabsorptive capacity was exceeded, producing marked hemoglobinuria [110] and intraluminal free iron release [110, 111]. These same events undoubtedly occur during myoglobinemia/uria, with the exception that myoglobin's small size (~17 kD) permits ready filtration without *in vivo* modification. The importance of these events to the pathogenesis of heme-induced proximal tubular injury is underscored by our current belief that heme protein toxicity is free iron induced. The evidence underlying this conclusion is discussed below.

A large body of *in vivo* and *in vitro* evidence support the concept that oxygen and non oxygen-based free radicals are critical mediators of extrarenal and renal injury [112–114]. Since iron is a transition metal which readily accepts and donates electrons, it greatly facilitates free radical production and may itself become a free radical [115–119]. Iron chelators, such as, DFO and 2,3-dihydroxybenzoic acid, have been shown to protect against seemingly diverse models of tissue injury. Iron-induced oxidant stress has become a generally accepted mediator of tissue damage [120–123]. Given this background information, and with Bunn's finding of intratubular cell iron release during hemoglobinuria, it seemed highly plausible that "catalytic" iron (that is, capable of promoting free radical reactions) could play a critical role in heme-induced nephrotoxicity. Two seminal studies substantiating this hypothesis were published simultaneously in 1988. Paller noted that DFO therapy mitigated three models of myohemoglobinuric renal injury: i.v. hemoglobin-induced nephrotoxicity, glycerol-induced ARF, and a combined renal ischemic/hemoglobinuric insult [124]. In the second study, Shah and Walker demonstrated that DFO and hydroxyl radical scavengers each protected against the glycerol ARF model [125]. Both of these studies reported a heme-induced increase in renal lipid peroxidation, as assessed by malondialdehyde (MDA) generation. This observation, and that hydroxyl radical scavengers completely prevented the MDA increment [125], strongly supported the concept that intrarenal heme iron accumulation induces hydroxyl radical formation and thus, oxidant renal damage.

In subsequent studies, Shah et al have provided further compelling evidence for the role of reactive oxygen species in the propagation of heme protein nephrotoxicity. Using the aminotriazole method, they first demonstrated increased H_2O_2 production in rat kidneys following myohemoglobinuria [126]. Since this is a "substrate" for hydroxyl radical formation via the iron catalyzed Fenton/Haber-Weiss reactions [115], this finding provided additional support for the authors' previously advanced hypothesis that hydroxyl radical is a mediator of heme-induced renal tubular damage. Next, they demonstrated that manipulations in the rat's renal glutathione status altered the expression of heme-induced tissue injury [127]. When rats were supplemented with glutathione, they were partially protected against glycerol-induced ARF. Conversely, when glutathione was depleted with L-buthionine-(S,R) sulfoximine, a worsening of ARF resulted. Since glutathione is a potent antioxidant, particularly in terms of eliminating H_2O_2, these studies firmly established the role of H_2O_2, and possibly its iron-catalyzed byproducts, in the induction of myohemoglobinuric ARF. Since H_2O_2 can cause iron release from intracellular sequestration sites (such as the cytochromes [111]), it may further promote oxidant stress by increasing the intracellular free, or "catalytic," iron burden.

In a series of elegant experiments, Nath et al substantially advanced our understanding of iron-induced oxidant stress in myohemoglobinuric renal injury by detailing the critical roles played by heme oxygenase and ferritin [128]. Since heme oxygenase (HO) degrades the porphyrin ring, it causes free iron release which then up-regulates intracellular ferritin expression, a key defense against iron-induced tissue damage. To demonstrate the involvement of these proteins in heme-induced ARF, HO inhibition was induced by Sn protoporphyrin administration: decreased ferritin expression and an exacerbation of the glycerol ARF model resulted [128]. Conversely, when ferritin expression was up-regulated by injecting rats with a subtoxic dose of hemoglobin 24 hours prior to glycerol injection, marked cytoprotection resulted [128]. We have recently found that acute HO inhibition (that is, without allowing time for increased ferritin expression) acutely protects against *in vitro* myohemoglobinuric proximal tubular cytotoxicity, undoubtedly because it inhibits heme iron release [106]. Thus, HO inhibition can have seemingly 'paradoxical' effects: on the one hand, it decreases ferritin expression, predisposing to iron toxicity; on the other hand, it can protect against heme toxicity by preventing heme iron liberation. Finally, we have demonstrated that DFO completely blocks myohemoglobinuric injury in our new *in vitro* model system [106]. Thus, when each of these studies are viewed together, they provide overwhelming evidence that iron is the key determinant of heme protein's cytotoxic effect.

Despite the above, at least three critical questions regarding the nature of heme iron-induced proximal tubular injury remain unresolved: *first*, which free radical(s) initiate and propagate the injury? *Second*, what are the site(s) and pathways of iron-based free radical production? *Third*, what are the critical biochemical targets, injury to which culminate in cell death? In regards to the first issue, Walker and Shah have provided compelling evidence that H_2O_2 and hydroxyl radical are important mediators of *in vivo* myohemoglobinuric ARF [126, 127]. However, we have been unable to demonstrate that either catalase or hydroxyl radical scavengers mitigate the *in vitro* expression of that injury directly at the proximal tubular cell injury [106]. This raises the possibility that these antioxidants might exert some of their protection via vascular or intraluminal effects. Of further note is that DFO's cytoprotective action *in vitro* has not correlated with any apparent decrease in hydroxyl radical production [106, 111, 129]. Thus, the

specific free radical(s) which propagate heme iron-induced injury remain in doubt [106, 118, 130, 131].

Regarding the second issue, a recent study from our laboratory provides evidence for the mitochondrion as a critical site of heme-induced free radical formation [132]. When heme laden proximal tubular segments were exposed to mitochondrial respiratory chain inhibitors, profound alterations in the expression oxidant cell injury (lipid peroxidation) resulted: blockade at site 2 (with antimycin A) or site 3 (with cyanide or hypoxia) completely prevented heme-induced MDA generation. Conversely, site 1 blockade dramatically increased oxidative damage. In contrast, these agents had virtually no effect on lipid peroxidation in normal tubules. Therefore, these results strongly point to the mitochondrial respiratory chain, in general, and its terminal portion, in particular, as a critical site of heme-induced free radical formation. Since free radicals tend to induce injury at the site of their formation, it would be predicted that mitochondrial damage would result. While a defect in cellular energetics was documented in the above study (a modest decrement in ATP/ADP ratios), total cellular ATP was reasonably well preserved. To summarize, these experiments suggest that while the mitochondria might fuel lipid peroxidation, they are probably not the critical subcellular target of iron dependent free radical attack.

Finally, the critical molecular targets of heme iron-induced oxidant stress remain largely unknown. While evidence has been presented that lipid, DNA, and protein oxidation may all be involved [106, 111, 124, 125, 133], linking oxidation of these cellular constituents to lethal cell injury has been exceedingly difficult to establish. As one example, although most studies have documented that lipid peroxidation accompanies heme protein nephrotoxicity, *in vitro* studies have not demonstrated that these two processes are directly related. For example, although addition of glutathione to heme loaded proximal tubules can protect them from lethal injury, it can also paradoxically increase lipid peroxidation [106]. Presumably this is because thiols can undergo autoxidation reactions, H_2O_2 production being a result [20, 134]. Conversely, addition of either ferrous and ferric salts to normal tubules induces massive and equal degrees of lipid peroxidation; however, only the former acutely induces cell death [129]. Given these complexities and the seemingly endless number of potential targets, it is unlikely that the specific, critical molecular lesion(s) underlying iron-induced cytotoxicity will soon become apparent.

Additional potential mediators of myohemoglobinuric ARF

For the sake of completeness, it should be recalled that rhabdomyolysis and hemolysis release more than just heme proteins into the circulation, raising the possibility that other cellular constituents might contribute to acute renal injury. As one example, clinically relevant degrees of hyperphosphatemia can markedly potentiate both experimental ischemic and nephrotoxic renal damage [135]. Hyperuricemia, another byproduct of excessive tissue catabolism, can contribute to cast formation and intratubular obstruction. Furthermore, since it is an organic anion, and since organic anion transport can potentiate experimental ischemic tubular injury [136], hyperuricemia could also impact on heme-protein associated ischemic tubular damage. Rhabdomyolysis can also trigger disseminated intravascular coagulation via tissue thromboplastin release, potentially causing intrarenal microthrombus formation. Thus, while heme proteinuria and renal ischemia may be the best documented mediators of rhabdomyolysis and hemolysis-associated renal injury, it is probably overly simplistic to assume that other tissue derived factors are not involved [137].

Prevention and therapy of heme pigment nephropathy

Seventy-five years of investigation into the pathogenesis of heme protein nephrotoxicity has led to rational strategies for preventing this form of ARF. The cornerstones of that therapy are as follows: (1) correction of hypovolemia and any attendant renal ischemia; (2) enhance the clearance of heme proteins from the circulation and the kidney; and (3) mitigate the direct adverse consequences of heme proteins on the proximal tubular epithelium.

Volume replacement therapy

Since intravascular volume status has such a profound impact on the development of experimental heme protein-induced ARF, vigorous intravenous fluid therapy, oftentimes administered with mannitol and $NaHCO_3$ (see below), is a mainstay in the early management of clinical rhabdomyolysis or severe intravascular hemolysis. Although no prospective clinical trials have proven their efficacy, retrospective analyses by Better and co-workers provide overwhelming support for their use [14, 58, 59]. These investigators compared the clinical outcomes of two groups of patients who developed crush syndrome during building collapses. In the first group of 7 patients seen in 1979, volume expansion was not instituted for up to at least six hours following patient extrication from the injury site, and in each case, ARF developed. In contrast, 7 patients who suffered similarly severe crush injuries in 1982 received i.v. saline therapy starting at, or even before, their extrication, and none developed ARF. An eighth patient in this latter series served as an "unintentional control" since on site fluid resuscitation was inadvertently omitted and ARF developed. Eneas, Schoenfeld and Humphries suggested that i.v. volume expansion may also be efficacious for non-traumatic rhabdomyolysis [138]. Thus, compelling clinical and experimental observations strongly support a role for aggressive, and most importantly, *early* fluid replacement (before substantial cast formation, heme endocytic uptake, and tubular necrosis occur). The critical questions are how much and what types of fluids should be administered? Based on Better's extensive personal experience with the crush syndrome, he recommends 1.5 liters/hr of normal saline as soon as a trapped limb is freed [16]. Once monitoring is possible, sufficient volume is administered to normalize systemic hemodynamics. Assuming that the patient can respond with an appropriate urine output, he then recommends ~12 liters per day of a mannitol/alkaline diuresis [16]. In contrast, considerably smaller volumes were used by Eneas et al in managing non-traumatic rhabdomyolysis [138]. Obviously, no firm rules exist, other than to "individualize" therapy.

Enhancing heme protein elimination

Volume expansion is critical to achieving this goal since improving GFR (reversing pre-renal azotemia, preventing ischemic tubular injury), inducing a diuresis, and diluting out intraluminal heme proteins lessen the risk of cast formation. The latter result may also lessen proximal tubular heme uptake, as previously discussed. Based on the pronounced protective effects of urinary alkalinization on experimental models of heme protein nephrotoxicity, systemic $NaHCO_3$ therapy is usually recommended, with

a goal of achieving a urine pH of > 6.5 [16, 138]. In our experience, the dosing requirements to achieve this goal vary considerably amongst patients. A particularly compelling case for systemic alkalinization can be made if severe myolysis, acidosis, and early renal dysfunction have produced severe hyperkalemia. However, it is worth noting that alkalemia may also predispose to hypocalcemia, which may already exist in patients with severe rhabdomyolysis due in part to hyperphosphatemia and skeletal muscle Ca^{2+} deposition [139]. While alkali-induced hypocalcemia may represent a theoretical risk, its occurrence could have dire consequences in crush syndrome patients. For example, were seizures to develop, further muscle injury and complications of pre-existent fractures could result. From a pathophysiologic standpoint, it should be noted that some of bicarbonate's protective effect stems from its serving as a non-reabsorbed solute which promotes a diuresis [90]. Whether alkalinization is useful once a brisk diuresis has been achieved with saline or diuretics (see below) remains unknown.

Mannitol has been repeatedly shown to protect against experimental myohemoglobinuric ARF, and hence, it is often added to saline/bicarbonate infusions. Multiple potential pathways for its protective actions exist: *first*, as a proximal acting diuretic, it is highly effective in facilitating heme protein/heme iron excretion [111]. Thus, a lessening of heme endocytosis and cast formation may result. *Second*, since it is a potent renal vasodilator, it may improve renal perfusion, possibly mitigating renal ischemic damage [140]; and *third*, since mannitol is a hydroxyl radical scavenger, it can theoretically decrease heme iron-induced oxidant stress. To test the relative importance of these actions, our laboratory studied the impact of mannitol on the glycerol model of ARF [141]. In that study, mannitol's protective action could be completely ascribed to a solute diuresis and increased heme excretion. This conclusion was based on the fact that a comparable solute diuresis induced by Na_2SO_4 infusion induced comparable protection. Furthermore, if the amount of heme protein which was excreted during the mannitol diuresis was returned to the rat by intravenous infusion, protection no longer resulted. Since mannitol is largely an impermeant solute, it is unlikely to effectively scavenge hydroxyl radical at an intracellular location. This consideration, plus the fact that Na_2SO_4 (which does not scavenge hydroxyl radical) conferred equal protection to mannitol, further suggests that mannitol's antioxidant property does not substantially contribute to its beneficial effect. Interestingly, although mannitol is a potent vasodilator, it may actually worsen, rather than improve, cellular energetics during the induction of ARF. For example, if administered immediately after renal ischemia [140] or during the early phase of glycerol-induced ARF [141], renal cortical ATP levels may abruptly decline. Although seemingly paradoxical, mannitol increases the metabolic cost (ATP consumption) of Na reclamation [142]. While under normal circumstances this has no effect on ATP concentrations, in the presence of concomitant tubular damage, a worsening of cellular energy depletion may result. Thus, improvements in renal perfusion and in cellular energetics need not be synonymous. Given these considerations, it is theoretically possible that furosemide might be a preferable diuretic. However, this remains only a theoretical possibility that remains to be tested. When mannitol is employed, it is critical to monitor serum osmolality to avoid a hyperosmolar state. This is particularly likely to occur in the setting of a substantially reduced GFR, limiting its filtration.

In the case of crush injuries, care of the damaged tissue may limit myoglobin release into the circulation, and hence decrease the renal burden. Better and Stein have suggested that mannitol may lower muscular intracompartmental pressures in experimental crush syndrome [16]. If a limitation of muscle necrosis were to result, this would be another potential benefit of mannitol administration. Surgical exploration, decompression, and fasciotomy have also been employed in an attempt to achieve this goal. However, Better and Stein have urged conservative management because of the risk of infections and possible limb loss [16]. In their view, surgery should only be undertaken when the limb is threatened by high intracompartmental pressures (>40 mm Hg, or < 30 mm Hg below diastolic pressure), confirmed by direct manometry. Finally, it is theoretically possible that extracorporeal elimination of circulating heme proteins could limit the renal heme burden. However, because of its size, myoglobin is poorly removed by either peritoneal dialysis or high permeability hemofilters [143]. Furthermore, even in the presence of severe ARF, circulating myoglobin levels fall exponentially with the cessation of its muscular release, probably because of hepatic and splenic uptake [143]. Thus, no compelling data indicating a role for extracorporeal heme protein removal (such as, by plasmapheresis) currently exists.

Limiting heme protein cytotoxicity

Each of the above interventions would be expected to limit heme protein proximal tubular injury since decreased proximal tubular heme endocytic transport should result. While the experimental literature has suggested a number of highly promising cytoprotective interventions, none have yet to undergo clinical trials. In our view, the most promising are the iron chelators, such as DFO, since they have been uniformly protective when used in divergent models of myohemoglobinuric tubular injury [106, 111, 124, 125]. Based on compelling *in vivo* data, glutathione supplementation also seems promising [127]. However, *in vitro* experiments suggesting that glutathione can exert pro- as well as anti-oxidant effects [106], and that Scaduto et al have found that glutathione may worsen experimental ischemic ARF [144], cause some reservation.

Future directions

Although an enormous amount of work has been directed at the prevention and treatment of early rhabdomyolysis-induced ARF, methods to enhance renal recovery in this syndrome have received little attention. Interventions to limit ongoing muscle injury may prove particularly rewarding since decreased renal heme exposure would result. Given the impressive experimental results obtained with growth factor therapy in diverse forms of toxic and ischemic renal injury [145], this approach may also prove useful. The impact of acute hemodialysis on the expression of heme protein nephrotoxicity also needs to be assessed. In light of the prolonged half life of intratubular cell iron [109], it seems possible that iron overload could predispose the tubular cell to recurrent damage in response to adverse dialytic events [146–148]. Finally, it must be recognized that in many ways, rhabdomyolysis-induced ARF is a societal and not just a medical disease. War, terrorism, trauma, poor building design and construction, inadequate earthquake

preparedness, and drug abuse remain leading causes of this syndrome. Hence, elimination of this disease will require social as well as medical interventions.

RICHARD A. ZAGER
University of Washington and the Fred Hutchinson Cancer Research Center, Seattle, Washington, USA

Acknowledgment

This work was supported by a research grant from the National Institutes of Health (DK 38432).

Reprint requests to Richard A. Zager, M.D., Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Room M621, Seattle, Washington 98104, USA.

References

1. HOLY BIBLE: Book of Numbers, Ch. 11, verses 31–35
2. BYWATERS EGL, BEALL D: Crush injuries with impairment of renal function. *Br Med J* 1:427–432, 1941
3. BRUMBACK RA, FEEBACK DL, LEECH RW: Rhabdomyolysis in childhood. A primer on normal muscle function and selected metabolic myopathies characterized by disordered energy production. *Ped Neurol* 39:821–858, 1992
4. GUYTON AC: Contraction of skeletal muscle (Chapt 6), in *Textbook of Medical Physiology* (8th ed), edited by GUYTON AC, Philadelphia, WB Saunders, 1991, pp 67–79
5. GUYTON AC: Excitation of skeletal muscle contraction: Neuromuscular transmission and excitation-contraction coupling (Chapt 7), in *Textbook of Medical Physiology* (8th ed), edited by GUYTON AC, Philadelphia, WB Saunders, 1991, pp 80–87
6. WALKER PM, LINDSAY TF, LABBE R, MICKLE DA, ROMASCHIN AD: Salvage of skeletal muscle with free radical scavengers. *J Vasc Surg* 5:68–75, 1987
7. KORTHIUS RJ, GRISHAM MB, GRANGER DN: Leukocyte depletion attenuates vascular injury in postischemic skeletal muscle. *Am J Physiol* 254:H823–H827, 1988
8. KORTHUIS RJ, SMITH JK, CARDEN DL: Hypoxic reperfusion attenuates postischemic microvascular injury. *Am J Physiol* 256:H315–H319, 1989
9. CARDEN DL, SMITH JK, KORTHUIS RJ: Neutrophil-mediated microvascular dysfunction in postischemic canine skeletal muscle: Role of granulocyte adherence. *Circ Res* 66:1436–1444, 1990
10. KLAUSNER JM, ANNER H, PATERSON IS, KOBZIK L, VALERI CR, SHEPRO D, HECHTMAN HB: Lower torso ischemia-induced lung injury is leukocyte dependent. *Ann Surg* 208:761–767, 1988
11. BAGGE U, AMUNDSON B, LAURITZAEN C: White blood cell deformability and plugging of skeletal muscle capillaries in hemorrhagic shock. *Acta Physiol Scand* 108:159–163, 1980
12. KLAUSNER JM, PATERSON IS, VALERI CR, SHEPRO D, HECHTMAN HB: Limb ischemia-induced increase in permeability is mediated by leukocytes and leukotrienes. *Ann Surg* 208:755–760, 1988
13. BELKIN M, LAMORTE WL, WRIGHT JG, HOBSON RW II: The role of leukocytes in the pathophysiology of skeletal muscle ischemic injury. *J Vasc Surg* 12:14–19, 1989
14. BETTER OS: The crush syndrome revisited (1940–1990). *Nephron* 55:97–103, 1990
15. COLLINS AJ: Renal dialysis treatment for victims of the Armenian earthquake. *N Engl J Med* 320:1291–1292, 1989
16. BETTER OS, STEIN JH: Early management of shock and prophylaxis of acute renal failure in traumatic rhabdomyolysis. *N Engl J Med* 322:825–829, 1990
17. GUHARAY F, SACHS F: Stretch-activated single ion channel currents in tissue-cultured embryonic chick skeletal muscle. *J Physiol* (Lond) 352:685–701, 1984
18. CHRISTENSEN O: Mediation of cell volume regulation by Ca^{2+} influx through stretch-activated channels. *Nature* 330:66–68, 1987
19. ODEH M: The role of reperfusion-induced injury in the pathogenesis of the crush syndrome. *N Engl J Med* 324:1417–1422, 1991
20. FREEMAN BA, CRAPO JD: Biology of Disease: Free radicals and tissue injury. *Lab Invest* 447:412–426, 1982
21. PENTILLA A, TRUMP BF: Extracellular acidosis protects Ehrlich ascites cells and rat renal cortex against anoxic injury. *Science* 185:277–278, 1974
22. BING OHL, BROOKS WW, MESSER JV: Heart muscle viability following hypoxia: Protective effect of acidosis. *Science* 180:1297–1298, 1973
23. GORES GJ, NIEMINEN AL, FLEISHMAN KE, DAWSON TL, HERMAN B, LEMASTERS JJ: Extracellular acidosis delays onset of cell death in ATP-depleted hepatocytes. *Am J Physiol* 255:C315–C322, 1988
24. ZAGER RA, SCHIMPF BA, GMUR DJ: Physiological pH. Effects on posthypoxic proximal tubular injury. *Circ Res* 72:837–846, 1993
25. OLERUD JE, HOMER LD, CARROLL HW: Serum myoglobin levels predicted from serum enzyme values. *N Engl J Med* 293:483–485, 1975
26. KNOCHEL J: Catastrophic medical events with exhaustive exercise: "White collar" rhabdomyolysis. *Kidney Int* 38:709–719, 1990
27. GABOW PA, KAEHNY WD, KELLEHER SP: The spectrum of rhabdomyolysis. *Medicine* 61:141–152, 1982
28. ARMSTRONG RB, WARREN GL, WARREN JA: Mechanisms of exercise-induced muscle fiber injury. *Sports Med* 12:184–207, 1991
29. KUIPERS H: Exercise induced muscle damage. *Int J Sports Med* 15:132–135, 1994
30. DUAN C, DELP MD, HAYES DA, DELP PD, ARMSTRONG RB: Rat skeletal muscle mitrochondrial $[Ca^{2+}]$ and injury from downhill walking. *J Appl Physiol* 68:1241–1251, 1990
31. LOPEZ JR, ROJAS B, GONALEZ MA, TERZIC A: Myoplasmic Ca^{2+} concentration during exertional rhabdomyolysis. *Lancet* 345:424–425, 1995
32. BELCASTRO AN: Skeletal muscle calcium-activated neutral protease (calpain) with exercise. *J Appl Physiol* 74:1381–1386, 1993
33. KNOCHEL JP: Mechanisms of rhabdomyolysis. *Curr Opinions Rheumatol* 5:725–731, 1993
34. ZAGER RA, GMUR DJ, BREDL CR, ENG MJ: Temperature effects on ischemic and hypoxic renal proximal tubular injury. *Lab Invest* 64:766–776, 1991
35. ZAGER RA, ALTSCHULD R: Body temperature: an important determinant of severity of ischemic renal injury. *Am J Physiol* 251:F87–F93, 1986
36. DMAISON L, GRYNBERG A: Cellular and mitochondrial energy metabolism in the stunned myocardium. *Basic Res Cardiol* 89:293–307, 1994
37. HONDA I, YONEMURA K: Acquired resistance to acute renal failure. *Kidney Int* 31:1233–1238, 1987
38. ZAGER RA: Heme protein-induced tubular cytoresistance: expression at the plasma membrane level. *Kidney Int* 47:1336–1345, 1995
39. ZAGER RA, BALTES LA, SHARMA HM, JURKOWITZ MS: Responses of the ischemic acute renal failure kidney to additional ischemic events. *Kidney Int* 26:689–700, 1984
40. ZAGER RA, IWATA M, BURKHART K, SCHIMPF B: Post-ischemic acute renal failure protects proximal tubular cells from O_2 deprivation injury. *Kidney Int* 45:1760–1768, 1994
41. FRÖHNER E: Über rheumatische Hämoglobinämie (toxämische hämoglobinurie Bollinger's) bein pferde und ihr verhältniss zur paroxysmalen hämoglobinurie des menschen. *Archiv Tierheilk* 10:296–327, 1884
42. MEYER-BETZ F: Beobachtungen an einem eigenartigen mit muskellähmungen verbundenen fall von hämoglobinurie. *Dtsch Arch Klin Med* 101:85–127, 1910
43. MCARDLE B, VEREL D: Myopathy due to defect in muscle glycogen breakdown. *Clin Sci* 10:13–35, 1951
44. POELS PJE, GABREËLS FJM: Rhabdomyolysis: A review of the literature. *Clin Neurol Neurosurg* 95:175–192, 1993
45. KNOCHEL JP: Rhabdomyolysis and myoglobinuria. *Ann Rev Med* 33:435–443, 1982
46. KNOCHEL J: Hypophosphatemia and rhabdomyolysis. *Am J Med* 92:455–457, 1992
47. KNOCHEL J, BILBREY G, FULLER T, CARTER N: The muscle cell in chronic alcoholism: The possible role of phosphate depletion in alcoholic myopathy. *Ann NY Acad Sci* 252:274–286, 1975
48. FERGUSON E, BLACHLEY Y, CARTER N, KNOCHEL J: Derangements of muscle composition, ion transport, and oxygen consumption in chronically alcoholic dogs. *Am J Physiol* 246:F700–F709, 1984
49. SINGHAL PC, KUMAR A, DESROCHES L, GIBBONS N, MATTANA J:

Prevalence and predictors of rhabdomyolysis in patients with hypophosphatemia. *Am J Med* 92:458–464, 1992
50. KNOCHEL JP, BARCENAS C, COTTON JR, FULLER TJ, HALLER R, CARTER MW: Hypophosphatemia and rhabdomyolysis. *J Clin Invest* 62:1240–1246, 1978
51. KNOCHEL J: Clinical effects of potassium deficiency on skeletal muscle, in *Potassium in Cardiovascular and Renal Medicine*, edited by WHELTON P, WHELTON A, WALKER W, New York, Marcel Dekker, 1986, pp 97–109
52. KNOCHEL J, SCHLEIN E: On the mechanism of rhabdomyolysis in potassium depletion. *J Clin Invest* 51:1750–1758, 1972
53. BYWATERS EGL, POPJAK G: Experimental crushing injury. *Surg Gynecol Obstet* 75:612–627, 1942
54. BYWATERS EGL, STEAD JK: The production of renal failure following injection of solution containing myohaemoglobin. *Q J Exp Physiol* 33:53–70, 1944
55. ZAGER RA: Heme protein-ischemic interactions at the vascular, intraluminal, and renal tubular cell levels: Implications for therapy of myoglobin-induced renal injury. *Renal Failure* 14:341–344, 1992
56. DUBROW A, FLAMENBAUM W: Acute renal failure associated with myoglobinuria and hemoglobinuria (Chapt 10), in *Acute Renal Failure* (2nd ed), edited by BRENNER BM, LAZARUS JM, New York, Churchill Livingstone, 1988, pp 279–293
57. ZAGER RA: Acute renal failure in the setting of bone marrow transplantation. *Kidney Int* 46:1443–1458, 1994
58. RON D, TAITELMAN U, MICHAELSON M, BAR-JOSEPH G, BURSZTEIN S, BETTER OS: Prevention of acute renal failure in traumatic rhabdomyolysis. *Arch Intern Med* 144:277–280, 1984
59. REIS ND, NICHAELSON M: Crush injury to the lower limbs. *J Bone Joint Surg-Am* 68:414–418, 1986
60. NOLAN JP, VENUTO RC, GOLDMANN S: Role of endotoxin in glycerol-induced renal failure in the rat. *Clin Sci Mol Med* 54:615–620, 1978
61. VOGT BA, ALAM J, CROATT AJ, VERCELLOTTI GM, NATH KA: Acquired resistance to acute oxidative stress: Possible role of heme oxygenase and ferritin. *Lab Invest* 72:474–483, 1995
62. SHULMAN LM, YUHAS Y, FROLKIS I, GAVENDO S, KNECHT A, ELIAHOU E: Glycerol induced ARF in rats is mediated by tumor necrosis factor. *Kidney Int* 43:1397–1401, 1993
63. BADR KF, KELLEY VE, RENNKE HG, BRENNER BM: Role for thromboxane A_2 and leukotrienes in endotoxin-induced acute renal failure. *Kidney Int* 30:474–480, 1986
64. ZAGER RA, PRIOR RB: Gentamicin and gram negative bacteremia: A synergism for the development of experimental nephrotoxic acute renal failure. *J Clin Invest* 78:196–204, 1986
65. PARILLO JE: Septic shock in humans. Advances in the understanding of pathogenesis, cardovascular dysfunction, and therapy. *Ann Int Med* 113:227–242, 1990
66. FURCHGOTT RF, JOTHIANANDAN D: Endothelial-dependent and independent vasodilatation involving cGMP: Relaxation induced by nitric oxide, carbon monoxide and light. *Blood Vessels* 28:52–61, 1991
67. NETO LM, NASCIMENTO OR, TABAK M, CARACELLI I: The mechanism of reaction of nitrosyl with met- and oxymyoglobin: An ESR study. *Biochem Biophys Acta* 956:189–196, 1988
68. SHARMA VS, TRAYLOR TG, GARDINER R: Reaction of nitric oxide with heme proteins and model compounds of hemoglobin. *Biochemistry* 26:3837–3843, 1987
69. BREZIS M, HEYMAN SN, DINOUR D, EPSTEIN FH, ROSEN S: Role of nitric oxide in renal medullary oxygenation. Studies in isolated and intact rat kidneys. *J Clin Invest* 88:390–395, 1991
70. AGMNON Y, PELEG H, GREENFELD Z, ROSEN S, BREZIS M: Nitric oxide and prostanoids protect the renal outer medulla from radiocontrast toxicity in the rat. *J Clin Invest* 94:1069–1075, 1994
71. SCHWARTZ D, BLUM M, PEER G, WOLLMAN Y, MAREE A, SERBAN I, GROSSKOPF I, CABILI S, LEVO Y, IAINA A: Role of nitric oxide (EDRF) in radiocontrast acute renal failure in rats. *Am J Physiol* 267:F374–F379, 1994
72. NOGUCHI S, KASHIHARA Y, IKEGAMI Y, MORIMOTO K, MORIMOTO M, NAKAO K: Insulin like growth factor-1 ameliorates transient ischemia-induced acute renal failure in rats. *J Pharm Exp Ther* 267:919–926, 1993
73. SABBATINI M, SANSONE G, UCCELLO F, DE NICOLA L, GILIBERTI A, SEPE V, MARGRI P, CONTE G, ANDREUCCI VE: Functional versus structural changes in the pathophysiology of acute ischemic renal failure in aging rats. *Kidney Int* 45:1355–1361, 1994
74. CHINTALA MS, CHIU PJ, VEMULAPALLI S, WATKINS RW, SYBERTZ EJ: Inhibition of endothelial derived relaxing factor (EDRF) aggravates ischemic acute renal failure in anesthetized rats. *Naunyn-Schmiedebergs Arch Pharmacol* 348:305–310, 1993
75. BOBADILLA NA, TAPIA E, FRANCO M, LOPEZ P, MENDOZA S, GARCIA-TORRES R, ALVARADO JA, HERRERA-ACOSTA J: Role of nitric oxide in renal hemodynamic abnormalities of cyclosporin nephrotoxicity. *Kidney Int* 46:773–779, 1994
76. AMORE A, GIANOGLIO B, GHIGO D, PERUZZI L, PORCELLINI MG, BUSSOLINO F, COSTAMAGNA C, CACACE G, PICCIOTTO G, MAZZUCCO G, SENA LM, COPPO R: A possible role for nitric oxide in modulating the functional cycosporine toxicity by arginine. *Kidney Int* 47:1507–1514, 1995
77. WRIGHT CE, REES DD, MONCADA S: Protective and pathological roles of nitric oxide in endotoxin shock. *Cardiovasc Res* 26:48–57, 1992
78. MAREE A, PEER G, SCHWARTZ D, SERBAN I, BLUM M, WOLLMAN Y, CABILI S, IAINI A: Role of nitric oxide in glycerol-induced acute renal failure in rats. *Nephrol Dial Transplant* 9(Suppl):78–81, 1994
79. VETTERLEIN R, HOFFMANN F, PEDINA J, NECKEL M, SCHMIDT G: Disturbances in renal microcirculation induced by myoglobin and hemorrhagic hypotension in anesthetized rat. *Am J Physiol* 268: F839–F846, 1995
80. ZAGER RA: Myoglobin depletes renal adenine nucleotide pools in the presence and absence of shock. *Kidney Int* 39:111–119, 1991
81. THOMPSON A, MCGARRY AE, VALERI CR, LIEBERTHAL W: Stroma free hemoglobin increases blood pressure and GFR in the hypotensive rat: Role of nitric oxide. *J Appl Physiol* 77:2348–2354, 1994
82. LIEBERTHAL W, LARAIA J, VALERI CR: Role of thromboxane in mediating the intrarenal vasoconstrction induced by unmodified stroma free hemoglobin in the isolated perfused rat kidney. *Biomat Artif Cells Immobil Biotechnol* 20:663–667, 1992
83. ZAGER RA, GAMELIN LM: Pathogenetic mechanisms in experimental hemoglobinuric acute renal failure. *Am J Physiol* 256:F446–F455, 1989
84. BAKER SL, DODDS EC: Obstruction of the renal tubules during the excretion of haemoglobin. *Br J Exp Pathol* 6:247–260, 1925
85. OLIVER J, MACDOWELL M, TRACY A: The pathogenesis of acute renal failure associated with traumatic and toxic injury. Renal ischemia, nephrotoxic damage, and the ischemuric episode. *J Clin Invest* 30:1307–1351, 1951
86. JAENIKE JR: The renal lesion associated with hemoglobinuria: a study of the pathogenesis of the excretory defect in the rat. *J Clin Invest* 46:378–387, 1967
87. OKEN DE, ARCE ML, WILSON DR: Glycerol-induced acute renal failure in the rat. I. Micropuncture study of the development of oliguria. *J Clin Invest* 45:724–735, 1966
88. RUIZ-GUINAZU A, COELHO JB, PAZ RA: Methemoglobin-induced acute renal failure in the rat. *Nephron* 4:257–275, 1967
89. JAENIKE JR: Micropuncture study of methemoglobin-induced acute renal failure in the rat. *J Lab Clin Med* 73:459–468, 1969
90. ZAGER RA: Studies of mechanisms and protective maneuvers in myoglobinuric acute renal injury. *Lab Invest* 60:619–629, 1989
91. MCQUEEN EG, ENGEL GB: Factors determining the aggregation of urinary mucoprotein. *J Clin Pathol* 19:392–1966
92. LEVI DF, WILLIAMS RC JR, LINDSTROM FD: Immunofluorescent studies of the myeloma kidney with special reference to light chain disease. *Am J Med* 44:922–933, 1968
93. POLLAK UE, ARBEL C: The distribution of Tamm Horsfall mucoprotein (uromucoid) in the human nephron. *Nephron* 6:667–672, 1969
94. ZAGER RA, COTRAN RS, HOYER JR: Pathologic localization of Tamm-Horsfall protein in interstitial deposits in renal disease. *Lab Invest* 38:52–57, 1978
95. WEINBERG JM: The cell biology of ischemic renal injury. *Kidney Int* 39:476–500, 1991
96. BONVENTRE JV: Mechanisms of ischemic acute renal failure. *Kidney Int* 43:1160–1178, 1993
97. FISH EM, MOLITORIS BA: Alterations in epithelial polarity and the pathogenesis of disease states. *N Engl J Med* 330:1580–1588, 1994
98. LEISER J, MOLITORIS BA: Disease processes in epithelia: Role of the

actin cytoskeleton and altered surface membrane polarity. *Biochim Biophys Acta* 1225:1–13, 1993
99. BADENOCH AW, DARMADY EM: The effect of stroma free hemoglobin on the ischaemic kidney of the rabbit. *Br J Exp Pathol* 29:215–223, 1948
100. YUILE CL, GOLD MA, HINDS EG: Hemoglobin precipitation in renal tubules. *J Exp Med* 82:36–374, 1945
101. LOWE MB: Effects of nephrotoxins and ischemia in experimental hemoglobinuria. *J Pathol Bact* 92:319–323, 1966
102. TRIFILLIS AL, KAHNG MW, TRUMP BF: Metabolic studies of glycerol-induced acute renal failure in the rat. *Exp Molec Pathol* 35:1–13, 1981
103. ZAGER RA, TEUBNER EJ, ADLER S: Low molecular weight proteinuria exacerbates experimental ischemic renal injury. *Lab Invest* 56:180–188, 1987
104. CORCORAN AC, PAGE IH: Renal damage from ferroheme pigments myoglobin, hemoglobin, hematin. *Texas Rep Biol Med* 3:528–544, 1945
105. PAGANO RE: Lipid traffic in eukaryotic cells: mechanisms for intracellular transport and organelle-specific enrichment of lipids. *Curr Opin Cell Biol* 2:652–663, 1990
106. ZAGER RA, BURKHART K, CONRAD DS, GMUR DJ: Iron, heme oxygenase, and glutathione: Effects on myohemoglobinuric proximal tubular injury. *Kidney Int* 48:1624–1634, 1995
107. ZAGER RA, BURKHART KM, CONRAD DS, GMUR DJ, IWATA M: Phospholipase A_2 induced cytoprotection of proximal tubules: Potential determinants and specificity for ATP depletion mediated injury. *J Am Soc Nephrol* (in press)
108. ZAGER RA, SCHIMPF BA, GMUR DJ, BURKE TJ: Phospholipase A 2 can protect renal tubules from oxygen deprivation injury. *Proc Nat Acad Sci USA* 90:8297–8301, 1993
109. BUNN HF, JANDL JH: The renal handling of hemoglobin. *II Catabolism J Exp Med* 129:925–934, 1969
110. BUNN HF, ESHMAN WT, BULL RW: The renal handling of hemoglobin. I. Glomerular filtration. *J Exp Med* 129:909–924, 1969
111. ZAGER RA: Combined mannitol and deferoxamine therapy for myohemoglobinuric renal injury and oxidant stress. Mechanistic and therapeutic implications. *J Clin Invest* 90:711–719, 1992
112. PALLER MS, HOIDAL JR, FERRIS TF: Oxygen free radials in ischemic acute renal failure in the rat. *J Clin Invest* 74:1156–1164, 1984
113. WALKER PD, SHAH SV: Evidence of the role of hydroxyl radical in gentamicin-induced acute renal failure in rats. *J Clin Invest* 81:334–341, 1988
114. SHAH SV: Evidence suggesting a role for hydroxyl radical in passive Heymann nephritis in rats. *Am J Physiol* 254:F337–F344, 1988
115. AUST SD, MOREHOUSE LA, THOMAS CE: Role of metals in oxygen radical reactions. *J Free Radical Biol Med* 1:3–25, 1985
116. HALLIWELL B, GUTTERIDGE JMC: Role of free radicals and catalytic metal ions in human disease: An overview. *Meth Enzymol* 186:1–85, 1990
117. CROSS CE, HALLIWELL B, BORISH ET, PRYOR WA, AMES BN, SAUL RL, MCCORD JM, HARMON D: Oxygen radicals and human disease. *Ann Intern Med* 107:526–545, 1987
118. MITSOS SE, KIM D, LUCCHESI BR, FANTONE JC: Modulation of myoglobin-H_2O_2-mediated peroxidation reactions by sulfhydryl compounds. *Lab Invest* 59:824–830, 1988
119. HALLIWELL B, GUTTERIDGE MC: Oxygen free radicals and iron in relation to biology and medicine: Some problems and concepts. *Arch Biochem Biophys* 246:501–514, 1986
120. FLIGEL SEQ, WARD PA, JOHNSON KJ, TILL GO: Evidence for a role of hydroxyl radical in immune-complex induced vasculitis. *Am J Pathol* 115:375–382, 1984
121. JOHNSON KJ, WARD PA, KUNKEL RG, WILSON BS: Mediation of IgA induced lung injury in the rat. Role of macrophages and reactive oxygen products. *Lab Invest* 54:499–506, 1986
122. WARD PA, TILL GO, HATHERILL JR, ANNESLEY TM, KUNKEL RG: Systemic complement activation, lung injury, and products of lipid peroxidation. *J Clin Invest* 76:517–527, 1985
123. WARD PA, TILL GO, KUNKEL R, BEAUCHAMP C: Evidence for role of hydroxyl radical in complement and neutrophil-dependent tissue injury. *J Clin Invest* 72:789–801, 1983
124. PALLER MS: Hemoglobin- and myoglobin-induced acute renal failure in rats: role of iron in nephrotoxicity. *Am J Physiol* 255:F539–F544, 1988
125. SHAH SV, WALKER PD: Evidence suggesting a role for hydroxyl radical in glycerol-induced acute renal failure. *Am J Physiol* 255: F438–F443, 1988
126. GUIDET B, SHAH SV: Enhanced in vivo H_2O_2 generation by rat kidney in glycerol-induced acute renal failure. *Am J Physiol* 257: F440–F445, 1989
127. ABUL-EZZ SR, WALKER PD, SHAH SV: Role of glutathione in an animal model of myoglobinuric acute renal failure. *Proc Nat Acad Sci USA* 88:9833–9837, 1991
128. NATH KA, BALLA G, VERCOLLOTTI GM, BALLA J, JACOB HS, LEVITT MD, ROSENBERG ME: Induction of heme oxygenase is a rapid and protective response in rhabdomyolysis in the rat. *J Clin Invest* 90:267–270, 1992
129. ZAGER RA, FOERDER CA: Effects of inorganic iron and myoglobin on in vitro proximal tubular lipid peroxidation and cytotoxicity. *J Clin Invest* 89:989–995, 1992
130. WINK DA, NIMS RW, SAAVEDRA JE, UTERMAHLEN WE JR, FORD PC: The Fenton oxidation mechanism: Reactivities of biologically relevant substrates with two oxidizing intermediates differ from those predicted for the hydroxyl radical. *Proc Natl Acad Sci USA* 91:6604–6608, 1994
131. YASUKAZU Y, KASHIBA K, NIKI E: Free radical-mediated oxidation of lipids induced by hemoglobin in aqueous dispersions. *Biochim Biophys Acta* 1201:165–172, 1994
132. ZAGER RA: Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. *Kidney Int* (in press)
133. UEDA N, SHAH SV: Endonuclease-induced DNA damage and cell death in oxidant injury to renal tubular epithelial cells. *J Clin Invest* 90:2593–2597, 1992
134. NATH KA, SALAHUDEEN AK: Autoxidation of cysteine generates hydrogen peroxide: Cytotoxicity and attenuation by pyruvate. *Am J Physiol* 264:F306–F314, 1993
135. ZAGER RA: Hyperphosphatemia: A factor that provokes severe experimental acute renal failure. *J Lab Clin Med* 100:230–239, 1982
136. ZAGER RA: Organic anion infusions exacerbate experimental acute renal failure. *Am J Physiol* 244:F47–F55, 1983
137. ZAGER RA: Precipitable tissue proteins can cause experimental acute renal failure. *Lab Invest* 59:798–808, 1988
138. ENEAS F, SCHOENFELD PY, HUMPHRIES MH: The effect of infusion of mannitol-sodium bicarbonate infusion on the clinical course of myoglobinuria. *Arch Int Med* 139:801–805, 1979
139. MERONEY WH, ARNEY GH, SEGAR WE, BALCH HH: The acute calcification of traumatized muscle with particular reference to acute post-traumatic renal insufficiency. *J Clin Invest* 36:825–832, 1957
140. ZAGER RA, MAHAN J, MEROLA AJ: Effects of mannitol on the postischemic kidney. Biochemical, functional, and morphologic assessments. *Lab Invest* 53:433–442, 1985
141. ZAGER RA, FOERDER C, BREDL C: The influence of mannitol on myohemoglobinuric acute renal failure: functional, biochemical, and morphological assessments. *J Am Soc Nephrol* 2:848–855, 1991
142. KNOX FG, FLEMING JS, RENNIE DW: Effects of osmotic diuresis on sodium reabsorption and oxygen consumption of kidney. *Am J Physiol* 210:751–759, 1966
143. WAKABAYASHI Y, KIKUNO T, OHWADA T, KIKAWADA R: Rapid fall in blood myoglobin in massive rhabdomyolysis and acute renal failure. *Intensive Care Med* 20:109–112, 1994
144. SCADUTO RC, VINCENT H, GATTONE II, GROTYOHANN LW, WERTZ J, MARTIN LF: Effect of an altered glutathione content on renal ischemic injury. *Am J Physiol* 255:F911–F921, 1988
145. HAMMERMAN MR: Therapeutic use of growth factor in renal failure. *J Am Soc Nephrol* 5:1–11, 1994
146. HAKIM RM: Clinical implications of hemodialysis membrane biocompatibility. *Kidney Int* 44:484–494, 1993
147. HAKIM RM: Effect of the dialysis membrane in the treatment of patients with acute renal failure. *N Engl J Med* 331:1338–1342, 1994
148. CONGER JD: Does hemodialysis delay recovery from acute renal failure? *Semin Dialysis* 3:146–148, 1990