Acute renal failure. II. Experimental models of acute renal failure: imperfect but indispensable

Molitoris, Bruce A., Joel M. Weinberg, Manjeri A. Venkatachalam, Richard A. Zager, Karl A. Nath, and Michael S. Goligorsky. Acute renal failure. II. Experimental models of acute renal failure: imperfect but indispensable. Am. J. Physiol. Renal Physiol. 278: F1–F12, 2000.—Acute renal failure (ARF) due to ischemic or toxic renal injury, a clinical syndrome traditionally referred to as acute tubular necrosis (ATN), is a common disease with a high overall mortality of ~50%. Little progress has been made since the advent of dialysis more than 30 years ago in improving this outcome. During this same period, a considerable amount of basic research has been devoted to elucidating the pathophysiology of ATN. The ultimate goal of this research is to facilitate the development of therapeutic interventions that either prevent ARF, ameliorate the severity of tubular injury following an acute ischemic or toxic renal insult, or accelerate the recovery of established ATN. This research endeavor has been highly successful in elucidating many vascular and tubular abnormalities that are likely to be involved in ischemic and toxic ARF. This information has led to impressive advances in the development of a number of different pharmacological interventions that are highly effective in ameliorating the renal dysfunction in animal models of ARF. Although these developments are exciting and promising, enthusiasm of investigators involved in this endeavor has been tempered somewhat by the results of a few recent clinical studies of patients with ATN. These trials, designed to examine the efficacy in humans of some of the interventions effective in animal models of ARF, have resulted in little or no benefit. This is therefore an important time to reevaluate the approaches we have taken over the past three to four decades to develop new and effective treatments for ATN in humans. The major goals of this review are 1) to evaluate the relevance and utility of the experimental models currently available to study ischemic and toxic renal injury, 2) to suggest novel experimental approaches and models that have the potential to provide advantages over methods currently available, 3) to discuss ways of integrating results obtained from different experimental models of acute renal injury and of evaluating the relevance of these findings to ATN in humans, and 4) to discuss the difficulties inherent in clinical studies of ATN and to suggest how studies should be best designed to overcome these problems.

acute tubular necrosis; ischemia; nephrotoxins; renal artery occlusion; cultured cells; isolated proximal tubules

1 Second in a series of invited articles on controversies related to acute renal failure.

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The kidney is a highly complex organ consisting of well-defined components that function in a highly coordinated fashion to allow for fine regulation of a myriad of interdependent processes. Novel and imaginative approaches have lead to the development of experimental techniques and models that now serve to direct the biochemical, cellular, and molecular approaches used to elucidate mechanisms of disease at cellular and subcellular levels. Only with this latter understanding can effective therapeutic modalities for renal diseases be designed. The purpose of this review is to emphasize the necessity of utilizing the vast array of complementary models available to further the understanding of acute renal failure (ARF), so as to facilitate the development of novel and effective therapeutic approaches for this disorder.

The ultimate goal of disease-oriented research remains the improvement of patient care and the reduction of morbidity and mortality. The process should start and end at the bedside with the initial disease description and testing of therapeutic options. Although the flow of questions begins with the patient, the elucidation of mechanistic information requires study of simpler models under physiological and pathological conditions. Utilization of animal, organ, tissue, cell culture, and reconstitution models is essential for obtaining the data necessary to fully understand disease processes (Fig. 1). Therefore, basic science research into fundamental cellular and molecular biology is critical to advance the knowledge of disease pathophysiology. Utilization of simpler models, however, requires verification of their observations in intact models. In vitro findings must be consistent with in vivo observations before extensive mechanistic studies proceed further. In addition, the efficient and effective utilization of information gleaned from simpler models requires understanding the strengths and weaknesses of each technique and model (Fig. 1).

One important concept that has greatly enhanced our understanding of the pathophysiology of ischemic ARF is that sublethal and reversible injury to renal tubular cells contributes substantially to renal tubular dysfunction. Prior to the development of this novel concept, it was generally believed that tubular dysfunction was predominantly the result of tubular cell necrosis. The concept that sublethal injury can contribute to renal dysfunction is a novel paradigm that provides an excellent example of how the exchange of ideas between clinical investigators and basic research, as well as the use of a number of different research models, can complement each other in advancing our understanding of human disease. I will use this example to elaborate.

One mechanism by which tubular injury causes a reduction in glomerular filtration rate (GFR) is by “backleak” of glomerular filtrate into the renal interstitium and renal venous system. The role of backleak in the functional abnormalities of ARF was first demonstrated in animal models (22). This idea was tested by Myers and his colleagues (59, 60) in humans with acute...
tubular necrosis (ATN), who confirmed that backleak is an important contributor to renal dysfunction in ischemic ATN. This represents one example of how ideas generated at the laboratory bench can be directly tested in humans.

The specific cellular mechanisms responsible for backleak subsequently became a focus of further research. Many investigators have utilized cultured tubular cells subjected to short periods of ATP depletion (using inhibitors of mitochondrial respiration) as a model of sublethal, reversible ischemic injury. Although ATP depletion induced in this way does not totally mimic ischemia, the observations proved consistent with the in vivo model and allowed investigators to proceed with mechanistic studies (17). Sublethal injury was demonstrated in these models to disrupt the actin cytoskeleton (40) and cause loss of cell polarity (55). The functional integrity of the junctional complex was also demonstrated to be reversibly impaired in cell monolayers subjected to the same model of sublethal injury (17, 46). These functional changes were demonstrated to be associated with alterations in the proteins associated with the tight junction such as ZO-1, which is believed to link occludin to the actin-based cytoskeleton (51). The cellular distribution of the tight junction proteins ZO-1 (5) and occludin (31, 83) and the adherens junction protein E-cadherin (6) have been reported to be altered during ATP depletion. Furthermore, ZO-1 and fodrin accumulated in cytoplasmic actin aggregates, indicating a role for actin cytoskeletal alterations in junctional complex alterations during ischemia (96).

It became important to apply this new knowledge to ischemic ARF in humans. The concept that sublethal injury to renal tubular cells is relevant to humans with ATN has been supported by a number of diverse studies. Racusen et al. (79) have demonstrated that patients with ATN shed viable renal tubular cells in their urine. Furthermore, Myers and coworkers (47) have recently designed studies to test the relevance of the cytoskeletal changes associated with ATP depletion in cell culture models to humans with ATN. They examined patients with ischemic tubular injury following renal transplantation, a relatively homogeneous population of patients with ATN. They have described a number of changes in the biopsies of these allografts that are consistent with many of the findings of the effect of sublethal injury induced by ATP depletion in cell culture models. For example, they have demonstrated that ischemic injury to the renal allograft in humans is associated with loss of polarity of the Na⁺-K⁺-ATPase (1), a finding comparable to that observed in sublethally injured cells in culture (55). They have also provided evidence in the same patient population that impaired tight junction integrity is associated with changes in the proteins associated with the junctional complex (47). Additional basic research is now required at the cellular level to elucidate the signal transduction pathways that are involved in the disruption of cytoskeleton and tight junction associated with ischemic renal tubular injury. These studies may lead to the design of novel and specific therapeutic approaches to the treatment of ATN.

Recent attempts to further elucidate the biochemical and molecular signaling events associated with the cytoskeletal changes induced by ischemia have necessitated a return to the use of the cell culture model of ATP depletion. The Rho family of GTP-binding proteins have been shown to regulate the assembly of actin-containing stress fibers and the organization and barrier function of tight junctions (67). Recent evidence, based on studies in which cultured cells were transfected with mutant forms of Rho-GTPase (either “kinase-dead” or constitutively active), suggests that the effects of ATP depletion on the cytoskeleton and tight junction are mediated via inhibition of Rho-GTPase activity (31, 53). These findings likely represent the beginning of the use of molecular biological techniques to probe the signaling events following ATP depletion that lead to dysfunction of sublethally injured cells. These types of studies have potential therapeutic implications, since tight junction disassembly and dysfunction following ischemic injury has the potential to contribute to backleak of glomerular filtrate between sublethally injured cells (17, 46, 57).

In summary, the example I have used represents the continuous cycle that should exist between ideas generated at the bedside and the testing of these hypotheses using simple experimental model systems (Fig. 1). It also demonstrates the importance of validating concepts derived from experimental models by testing them by devising appropriate clinical studies. Continuing progress in patient care has always been dependent upon interactions between basic and clinical scientists. The combined use of experimental models to develop mechanistic hypotheses and the testing of these hypotheses in humans continues to be a successful strategy that drives medical progress. This paradigm should represent the basis for the development of novel therapeutic approaches to the treatment of ARF.

B. A. Molitoris

THE PATHOGENESIS of ischemic and related forms of ARF involves a complex interplay of vascular and tubular factors that need to be defined individually as well as in terms of their interactions with each other for the entire process to be understood. Although tubules make up much of the mass of the renal cortex, the heterogeneity of metabolic function and susceptibility to injury of different nephron segments and the difficulty both of delivering well-defined insults in vivo and dynamically assessing metabolic changes in the intact organ makes it necessary, as in most other areas of cell biology, to use isolated cells for studies of the cellular pathophysiology of proximal tubular injury.

It is well appreciated that cells in culture undergo a variety of phenotypic changes driven in large part by the adaptations necessary to survive in vitro as well as by a shift to a more proliferative mode that characterizes that setting. These changes are particularly problematic for studies of the pathogenesis of injury to the
proximal tubule. Proximal tubular cells change from their normal dependence on oxidative, mitochondrial metabolism to glycolysis under culture conditions (94) and, as a result, become less susceptible to hypoxic injury. Cultured tubular cells also undergo considerable structural changes that include simplification of both their apical and basolateral membrane compartments (46) that affect cellular characteristics prominently involved in the behavior of the tubules during ARF in vivo.

For these reasons, freshly isolated proximal tubules have been favored by a number of investigators for studies of the cellular biology of proximal tubule injury during ARF (3, 28, 32, 34, 85, 93, 101, 105, 121). In vitro preparations of isolated proximal tubules have been widely used for a variety of metabolic and physiological investigations for over three decades and have also been employed extensively for studies of tubular cell injury during the past 16 years (32, 34, 93, 101, 105). This preparation has been valuable for both metabolic and structural investigations, because it retains the biochemical properties of the in vivo state as well as a high degree of structural integrity as well as the highly polarized and fully differentiated features of the normal proximal tubular epithelium.

As typically prepared and studied, the tubules exhibit a substantially increased sensitivity to ATP depletion-induced injury relative to that observed in vivo. Severe hypoxia or anoxia results in necrosis of a majority of cells within 15–30 min (45, 66, 93, 101, 105). The search for mediators and modifiers of this behavior led to recognition of the major cytoprotective effect of acidosis on tubules (101) and the discovery of the powerful role of glycine in promoting resistance to lytic plasma membrane damage, which is expressed in multiple tubule cell types as well as endothelial cells and hepatocytes (3, 28, 52, 88, 102, 104, 126). Simply replacing glycine that is lost during isolation or incubation under energy deprivation conditions to concentrations of 2–4 mM (levels that are at the low end of those normally found in tissues), confers a high degree of resistance to lytic membrane damage (99, 103–106). This permits full progression of sublethal structural changes as they occur in vivo where glycine remains relatively abundant during many forms of injury. Concomitant measurements of metabolic alterations in the glycine-protected tubules have allowed assessment of the full extent of changes in parameters such as cytosolic free calcium (105), phospholipid hydrolysis (99), actin polymerization (66, 89), and nitric oxide production (112) uncomplicated by nonspecific cellular disruption. The information thus derived is beginning to provide a more accurate view of the extent to which these factors contribute to ATP depletion-induced injury in tubular cells.

The use of freshly isolated tubules has also allowed the assessment of early recovery events, since both metabolic and structural changes can reverse during reoxygenation after hypoxia (104, 107). As with studies during hypoxia/ATP depletion, the presence of glycine and the use of acidic pH during hypoxia allows recovery from injury to be studied in a more relevant context. In this regard, recent work has revealed the development of a mitochondrial functional deficit that becomes severe after durations of hypoxia greater than 30 min and markedly limits both metabolic and structural recovery despite continued protection against lytic plasma membrane damage by glycine (107, 108). This model has the potential to provide additional opportunities in the future for clarifying long-standing unresolved issues about the role of compromised energetics during tubule cell injury and for developing approaches that alleviate the lesion.

In summary, freshly isolated proximal tubules provide a powerful system for defining cellular events during ARF, because they completely retain the fully differentiated phenotype as it exists in vivo but are accessible for direct experimental manipulations and measurements. By studying them in the presence of glycine, the normal resistance to lytic membrane damage seen in vivo is preserved, and a variety of sublethal events during both hypoxia and reoxygenation become amenable to mechanistic analysis.

The outcome of ARF in vivo is a function of the interaction of multiple cell types in the tubular and vascular compartments that must ultimately be understood at the whole tissue level and is not predictable from the study of the behavior of a single cell type (Fig. 1). Nonetheless, mechanistic information about the determinants of the responses of the individual cellular components involved remains critical for a rational and complete understanding of the process and attempts to modify it.

J. M. Weinberg

THE COMPLEX INTERACTIONS of several hemodynamic, humoral and toxic factors in the pathogenesis of human ARF have previously been reviewed (14, 49, 61). The need for whole animal studies to unravel these complexities and develop therapeutic modalities has been partially met by the development of “single insult” models for ARF induced by ischemia (15, 24), drugs and toxins (4, 97), radiocontrast agents (37), and rhabdomyolysis (62, 114). However, these models fall far short of reproducing the morphological features of ATN in humans. Attempts to develop such models more representative of the human disease we call ATN continues to elude investigators.

Ischemic ATN in humans occurs most often in the context of volume depletion, sepsis, and shock. The major renal histological findings in humans with ischemic ATN are focal areas of tubular necrosis. Recent evidence suggests that tubular cells between the relatively sparse areas of cell necrosis (so-called by some pathologists as “skip” areas) are often sublethally injured (1, 47) in humans with ATN. Experimental work at a cellular level (56) as well as in humans with ATN (1, 47, 76, 77, 79) has provided substantial evidence that sublethal injury can contribute substantially to tubular dysfunction in ischemic ARF (56). Another distinctive histological feature of ischemic ATN is the
presence of casts, predominantly localized in distal nephron segments (39, 68, 71, 72, 78, 90). The casts seen in ATN may contribute to renal failure by causing intratubular obstruction.

Attempts to reproduce these characteristic pathological features of ischemic ATN in experimental models have not succeeded. The most common cause of ischemic ATN in humans is shock due to hypovolemia or sepsis. However, severe and prolonged hypotension does not induce renal injury in rats (120). A suitable model of sepsis-associated ARF in rats is also not available. Many investigators have found it convenient to study reversible rat and mouse models of ischemic tubular damage caused by complete occlusion of the renal artery (RAO) for various time periods. A wealth of important information has been obtained using this model. However, there are serious questions regarding the adequacy of the RAO model as a paradigm for human ischemic ARF.

ARF in rats following RAO is characterized by extensive necrosis of proximal tubules, the distribution and extent of which vary with the ischemic interval; the distal nephron is less affected, with mild damage in thick limbs of the loops of Henle and focal apoptosis of cells in distal convoluted tubules and collecting ducts (9, 24, 30, 44, 50, 82, 86, 87). In distinct contrast, frank tubular necrosis is far less extensive in humans with ischemic ATN than in the rat following RAO. In humans, morphological injury is usually subtle and focal, affecting both proximal and distal tubules (39, 68, 72, 78, 90). However, there are some important similarities between the RAO model of ischemic ARF and human ATN. Pathological similarities include injury to the proximal brush border, the predilection for the most severe injury to occur in the proximal straight tubules (S3 segments), and the presence of cast formation. Functional similarities between ischemic ATN and the RAO model of ARF include the severe reduction in GFR and the reversibility, in most cases, of tubular injury with recovery of renal function.

When duration of ischemia is insufficient to cause necrosis in the reperfused rat kidney, a variety of sublethal structural alterations can be seen (58, 110). These changes may lead to impaired vectorial transport across tubular epithelium. It is noteworthy that studies of human ARF following transplantation have emphasized the role played by sublethal tubular injury similar to that seen in the rat as a pathogenetic factor causing sustained preglomerular vasoconstriction and filtration failure through tubuloglomerular feedback mechanisms (1, 47).

In the rat RAO model, persistent vasoconstriction and cellular swelling cause poor blood flow in the deep cortex and outer medulla, after the arterial clamp is released (26, 27, 54, 70, 92). Reflow may be so poor that medullary infarction results. These considerations suggest that persistent medullary ischemia exacerbates the injury caused by the original ischemic insult in this model. Indeed, improvement of blood flow by increase of perfusion pressure, reduction of hematocrit, volume expansion with saline, cell-impermeant solutes, or contralateral nephrectomy dramatically decreases injury and accelerates recovery following release of RAO in the rat (24, 26, 27, 54, 70, 92, 111, 117, 123). Many interventions that reduce the severity of ARF in animals, such as atrial natriuretic peptide, endothelin antagonists, and anti-inflammatory agents, probably exert their beneficial effect predominantly by improving renal perfusion (11, 19, 20, 29, 33, 41, 49, 64, 65, 74, 100). Even the therapeutic effect of interventions not generally recognized to have vascular effects, such as growth factors, may be mediated, in part, by intrarenal vasodilation (19, 25, 36, 38). Ischemic renal damage leads to vigorous inflammatory responses, which aggravate injury by vascular as well as parenchymal effects (12, 18, 75, 95). Thus, in the setting of ischemic and inflammatory injury complicated by persistent reperfusion defects, anti-inflammatory treatments as well as other agents may benefit the kidney by direct or indirect biochemical effects that lead to improvement of renal blood flow following the release of RAO.

Therefore, the possibility exists that the effects of agents with therapeutic benefit in the rat RAO model are mediated in large part by improved hemodynamics rather than specific biochemical mechanisms related to epithelial injury or repair. Should this be true, there would be need for caution in relating the results obtained using rats to human disease. Such extrapolations will demand that the hemodynamic response of the human kidney to ischemic insults is comparable in some degree to that of the rat. At least in dogs, we known that this is not so; juxtamedullary and total cortical or renal blood flow are not reduced following clamp release, even after RAO for 180 min (48, 80). This may explain why 120–180 min of warm renal ischemia is required to ensure lack of survival in dogs (35), relative to 90–120 min in rabbits (7) and about 60 min in rats and mice. In this regard, human kidneys resemble those of dogs; periods of warm ischemia up to 75 min are tolerated by transplants without giving rise to significant ARF (43). Differences between species in regard to hemodynamic responses to ischemic insults could possibly be related to variations of relative medullary thickness, a determinant of concentrating ability (84). Sluggishness of circulation and greater complexity of the vasculature in more voluminous and elongated medullae of kidneys in some species such as rats and mice might conceivably predispose them to ischemia caused by reflow abnormalities.

Experimental models useful for examining nephrotoxic causes of ARF have also been difficult to develop. The administration of aminoglycosides, radiocontrast media, or endotoxin, in the absence of other simultaneous renal insults, does not reliably induce ARF in rats. Some investigators have stressed the need for combined insults to mimic the process of ATN in humans. For example, nephrotoxicity of gentamycin toxicity is enhanced in the setting of gram-negative bacteremia (118, 124). Similarly, Brezis and colleagues (37) have demonstrated that radiocontrast media will induce renal injury in rats subjected to a combination of
DESPITE MAJOR ADVANCES in our understanding of pathogenetic events in ischemic and toxic ARF, clinical outcomes of this disease, as well as therapeutic approaches to it, have not substantially changed over the past 25 years. There are a number of complex explanations for this paradox. As examples: 1) an increasing ability to support critically ill patients increases the incidence of ARF, but the frequent, concomitant presence of multiorgan failure decreases chances for recovery; 2) documenting the onset of critical acute tubular injury is difficult (i.e., there are no “renal angina” equivalents), hindering the effective prophylaxis; 3) although we neatly categorize acute tubular injury as either “ischemic” or “toxic” in origin, clinical ARF likely has a multifactorial basis, as Venkatachalam has pointed out in his comments, above. For example, clinical myoglobinuria is generally well tolerated unless concomitant renal “ischemia” exists (8). Similarly, aminoglycoside-induced ARF is critically dependent on factors inherent to the septic state (e.g., endotoxemia) (124). Hence, although we may choose to take a “reductionist” experimental approach to gain specific mechanistic insights, we may be underestimating the complexity of the clinical disease, thereby slowing therapeutic progress.

The case of ischemic ARF illustrates this dilemma. Our usual logic in studying this disease flows from the following paradigm: 1) since transient hypotension precedes the onset of clinical ischemic ARF, it is assumed that severe renal hypoperfusion resulted; 2) this renal hypoperfusion is next assumed to have seriously compromised renal oxygen delivery/mitochondrial respiration, thereby producing critical ATP depletion (<10% basal values; Ref. 98); 3) this energy depletion initiates a complex injury cascade, culminating in tubular necrosis, apoptosis, and tubular cell detachment (9, 49, 50, 102); and 4) the resulting architectural abnormalities produce tubular obstruction, backleak, and filtration failure.

To experimentally recapitulate this presumed sequence of events, we investigators have relied predominantly on transient but complete cessation of renal blood flow, usually induced by RAO, a model that has already been described in some depth by Venkatacha-
conclusions about in vivo ischemic injury based solely or in vivo tubules. The potential hazards of drawing proliferating "proximal tubule" cells with marked mor-

drial respiration and glycolysis. There is no question that critical insights have resulted from these types of experiments. However, it seems prudent to remain open to the possibility that the above paradigm and the investigations flowing from it could potentially lead us astray by generating data that are not necessarily relevant to clinical postischemic ARF.

The following specific points illustrate this concern. First, as pointed out by Venkatachalam, although most clinical studies define "ischemic" ARF as that which follows a transient episode of hypotension (80–100 mmHg systolic), far more profound and prolonged forms of experimental shock (e.g., <50 mmHg for 2–3 h) have consistently failed to induce sufficient tubule energy depletion to induce "postischemic" ARF in rats (69, 73, 116). To produce tubular necrosis and concomitant ARF in rats, renal perfusion pressure must be reduced to ~20 mmHg (120). Such severe renal hypoperfusion could only be induced by near complete suprarenal aortic ligation, since attempts to lower systemic pressure to ~20 mmHg caused instant death. Thus these observations raise serious questions as to whether shock alone can cause ARF. Second, even if we assume that hypotension-induced tubule energy deple-
tion, per se, causes severe renal damage, it is important to note that this injury may not be closely simulated by the RAO model. For example, RAO (but not severe renal hypoperfusion) causes marked microvascular injury (120). Microvascular injury can inhibit vascular flow, prolonging the ischemic period. Furthermore, complete interruption of blood flow, but not hypoperfu-
sion, prevents renal efflux and influx of critical determi-
nants of tissue injury (e.g., CO2/HCO3, Ca2+, adenosine, and substrates for free radical generation such as hypoxanthine/xanthine) (120). Given these types of differences between complete vascular occlusion and hypoperfusion, some of our assumptions about the pathogenesis of ischemic ATN that have been based on results form the RAO model may not be correct. Third, when we use in vitro proximal tubules to gain mechanistic insights, we must remember that these tubules sustain substantial isolation damage. For example, 10–15% of cells in these preparations are lethally injured (as assessed by lactate dehydrogenase leakage). This means that the remaining still viable cells also have substantial sublethal damage, which could poten-
tially set the stage for potentially misleading results. Fourth, when we finally move to cultured tubular cells to avoid this "isolation injury," we encounter rapidly proliferating "proximal tubule" cells with marked mor-
morphological and metabolic differences to freshly isolated or in vivo tubules. The potential hazards of drawing conclusions about in vivo ischemic injury based solely on results obtained with cultured cells are underscored by Weinberg. Finally, it must be kept in mind that in vitro studies using both isolated tubules and cultured cells differ in critical ways from in vivo ischemia. As just one example, tissue acidosis occurs during ischemia in vivo, an event that substantially mitigates necrotic cell death (10, 125). In contrast, "ischemic" injury in vitro is generally conducted at physiological pH, which prevents the expression of the cytoprotective effect of acidosis (125). Furthermore, "ischemia" that is simu-
lated by the use of mitochondrial inhibitors ("chemical anoxia") is usually induced in the presence of normal oxygen tensions. Depending on the type of mitochon-
drial inhibitor used, this oxygen can dramatically fuel free radical generation (113). Thus tubular injury due to "chemical anoxia" is probably the result of the combined effects of ATP depletion and oxidant injury.

Given the limitations inherent to each of these experimental approaches, the suggestion of B. Molitoris, that we seek correlations between in vivo and in vitro models of tubular injury, seems absolutely essential. If congruous results can, in fact, be documented in a "vertical" series of experiments (from whole animal to isolated tubules to cell culture), then the likelihood of these data being clinically relevant data is greatly enhanced and increases opportunities for therapeutic advances. One illustrative example of this approach comes from multiple laboratories that have studied ARF due to myoglobinuria. This form of renal injury has been shown to be dependent on the availability of free "catalytic" iron in multiple models including cell culture, freshly isolated heme-loaded isolated prox-
imal tubules, and in multiple experimental models of in vivo heme protein-induced ARF (115). Given these observations, it is not surprising that iron chelation therapy (desferoxamine) confers striking protection against in vivo models of myoglobinuric ARF (115). Finally, to further our chances for therapeutic advance, it seems prudent to continually question whether our in vivo ARF "models" do, in fact, accurately model the disease that we wish to target. If not, then clinical trials with new therapeutics may be precluded from success from the outset.

R. A. Zager

DISEASE PROCESSES can be interrogated by disease models in ways that are neither possible nor permissible in a clinical setting. The use of in vitro and in vivo models that are faithful to the disease under consideration and amenable to manipulations required to pursue testable hypotheses, contributes invaluably to the un-
derstanding of the pathogenesis of disease and the delineation of potential therapeutic approaches.

Yet the application of such models should always be tempered by certain considerations. The first pertains to an inherent irony implicit in their use: on the one hand, disease models are intended to promote understanding of human disease, and on another, they can only be properly devised and applied insofar as the defining features of the relevant human disease are understood.
To the extent that such understanding is incomplete, the relevance of the employed model may be called into question. The second consideration relates to the first and emphasizes the need for continual cognizance of the intrinsic limitations of the utilized model and its points of divergence from the human disease. For example, the most widely applied in vivo model of human ischemic ARF makes use of a single intervention in the rat, namely, temporary and total RAO, whereas human ischemic ARF is commonly antedated by renal hypoperfusion and is often multifactorial, reflecting as it does the contributing effects of nephrotoxins and sepsis. Another difference between the RAO model of ARF and ATN is that cellular necrosis and other gross histological evidence of cell injury are abundant in the rat model but uncommonly seen in human ischemic ARF (78). A final consideration pertains to the tradeoff that exists with regard to the complexity of the model: as a disease model is simplified so as to examine with greater clarity the impact of a given variable, the likeness to the original disease may weaken, and this loss of resemblance may mitigate what is gained in resolution (Fig. 1).

The accompanying commentaries of Molitoris and Weinberg provide trenchant treatment of contrasting, yet complementary, themes in the use of models of ARF; the former emphasizes the need for interdigitation of clinical and experimental observations as a safeguard for relevance of findings from disease models, whereas the latter demonstrates how a bona fide model can provide novel insights into the cell biology of renal injury. Using tight junction dysfunction as a determinant of backleak in ARF as an example, B. Molitoris illustrates how clinical observations provide the basis for experimental analysis, and insights so garnered, in turn, stimulate a search for substantiation in human disease. This repetitive cycling between clinical observations and basic research ensures that experimental models remain true to the human condition.

Of the spectrum of models utilized in the study of ARF, each has its own mix of virtues and limitations, and the contributions of one of them, the freshly isolated tubule preparation, is discussed by Weinberg. As pointed out, the properties of this model straddle the divide between in vitro and in vivo preparations in that it is amenable to manipulations employed in vitro while it retains many of the characteristics of proximal tubules evident in vivo, thereby making it an appealing model in studies of renal injury (104, 106, 107, 109, 119, 122). Among the insights provided by this model is the recognition of the endogenous cytoprotection afforded by the high concentrations of glycine normally present within cells (104, 106). This discovery grew out of the realization that tubules studied in glycine-free media acquire susceptibility to injury occasioned by the depletion of intracellular stores of glycine. This finding is particularly instructive to the present discussion in that this loss of cellular glycine could have been easily dismissed as a confounding artifact of this preparation, and thus grounds by which to decry its merit rather than emphasize its value, and yet, this finding, perci-

ently pursued, ultimately led to the discovery of glycine as a cytoprotective agent against diverse insults and as an endogenous intracellular protectant against plasma membrane porosity (104, 106). Thus unique contributions to the cell biology of ARF occasionally result from what could have been considered a shortcoming of a disease model; these findings emphasize scientific rigor with which a disease model is studied as a critical determinant of the ultimate value of the model. Moreover, it is unlikely that these contributions would have arisen from an in vivo model and even less so from studies of human ARF.

In summary, disease models best serve the cause of understanding ischemic and nephrotoxic forms of human ARF when these models continually harken back to clinical observations for substantiation and validation. The limitations in our understanding of human ARF, in part, frustrate our attempts to accurately model these disorders and induce us to reach out to a multiplicity of models. Each model, however limited, nonetheless provides a unique perspective on these diseases and can offer up novel and wonderful insights on the pathobiology of acute renal injury. Although the true significance of some of these insights in their nascency may be difficult to evaluate, with time and with the steady accretion of understanding of human disease, these insights will ultimately be assigned their merited position within the context of the human disorder. Moreover, out of the rich diversity of available models, each illuminating some aspect of the human condition, a composite image emerges; and by continuing to explore these models in consultation with clinical observations, this image can be steadily refined, thereby bringing us that much closer to the truth.

K. A. Nath

Since the time Francis Bacon substituted philosophical dogmas of the past with the pragmatic challenge to all testable hypotheses, the theory of gnosticism has been thoroughly developed. Any investigative process begins with the creation of models, first simplistic and later on increasingly more sophisticated, to get answers to defined questions. Depending on the specific question, the actual model can be changed, but the rule of ascent from simple to complex remains. It asserts that findings and conclusions made in a simplified model system should be applicable to the more complex model; if this is not the case, a model and/or a hypothesis should be revised.

In the field of biological sciences, this translates into a view that the final litmus test for any hypothesis will be represented by its validity in humans. From this standpoint, half a century of research into the therapy of ARF should awaken us to the alarming situation: all clinical trials have resulted so far in a failure of approaches that in simpler model systems seemed to be effective. The situation is so ambiguous that experienced nephrologists still prefer to treat patients with ATN conservatively, providing only symptomatic relief
and hemodialysis as needed. The efficacy of clinically available modalities such as dopamine (21) and diuretics (such as Lasix and mannitol) (91) have turned to be equivocal at best. Clinical trial of therapeutic modalities such as atrial natriuretic peptide (2) or IGF-I (25), which have been shown to be highly effective in ameliorating renal dysfunction in rat models of ARF, have shown little more than borderline efficacy. Why is there such a discrepancy between the efficacy of pharmacological interventions in experimental models of ARF and in humans with ATN? I will discuss several factors I believe responsible for this problem.

First, the dialectics of ascent in disease models, as described and illustrated by Molitoris, has not entered the practice of many investigators. Molitoris offers the view that advantages and disadvantages of each model will be canceled when an idea is cross-tested using different approaches. His review provides a valuable list of strengths and weaknesses for most experimental systems used in studies of acute renal injury. In addition, Weinberg presents a detailed analysis of a single-model approach: isolated renal tubules. In my opinion, a model should not turn into a dogma, and it is unwarranted to earmark one experimental approach in favor of others. There is no “one model fits all,” and the reluctance to detach from a single, albeit well-established approach, while building on its strengths, may uncontrollably amplify its weaknesses. The second problem that haunts many an investigative effort, in my opinion, is a belief that the syndrome of ARF is, in general, uniform regardless of the inciting causes, and therefore, the results obtained in one model system can be extrapolated to others. In fact, however, what is true for the contrast-induced injury may be false for ischemic injury and vice versa. The third fallacy lies in a misguided effort to institute the same therapeutic agent at any time point of the natural history of the syndrome. There is no “magic bullet” for all times. The process is dynamic, and it should be studied as such. Consequently, therapeutic modalities which are appropriate at the onset may be mistargeted when the injury has been established.

In short, I believe that the major weakness of available models of ARF are: 1) insufficient attempts to emulate the clinical condition; 2) the need to test one model (e.g., cultured cells) in other models (e.g., the whole animal); and 3) the need to introduce into experimental studies some criteria used in clinical trials, such as the importance of timing of interventions, the use of the double-blind approach, and the testing of combinations of therapeutic modalities.

Clinical trials also need to be improved. Each pharmacological should have a defined optimal target in terms of disease processes, timing of intervention, necessity of supportive medications, etc. The end points of trials need to be more realistic. It is irrelevant to monitor the effect of a medication on the mortality rate in patients likely to survive the episode of ARF even when conventional therapies for ARF are used. Other criteria such as need for dialysis and rapidity of recovery of renal function are more relevant in this situation. It is equally naive to believe that improved management of ARF can improve the outcome of critically ill, elderly patients and patients with multiorgan failure. I believe that the major weaknesses of today’s design of clinical trials are: 1) the choice of outcome variables, e.g., survival and/or surrogate end points; 2) the use of combinations of medications; 3) improved appreciation of relevance of the timing of administration of the intervention; and 4) improved appreciation of diversity of pathophysiological mechanisms acting during different stages of ARF secondary to various etiologies.

On the basis of these considerations, I propose two major revisions in our approaches to both experimental models of ARF and clinical trials of ATN: first, that we revise the models and experimental strategies employed to study the pathophysiology of ARF so that the incremental usage of multiple disease models gains a broader base and, second, that clinical trials are designed in a fashion that brings more reason and pathophysiological understanding into their conduct. This may help to reconcile results of our experimentation in cells and animals with our findings in humans.

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