Free Rad. Res., Vol. 35, pp. 341–365
Reprints available directly from the publisher
Photocopying permitted by license only

Review

Implications for the Role of Endogenous Nitric Oxide Inhibitors in Hemodialysis Hypotension*

ELLEN S. KANG[a,b,†], SERGIO R. ACCHIARDO[c] and ANDREW H. KANG[c,d]

[a]*Departments of Pediatrics,* [b]*Pharmacology,* [c]*Medicine, University of Tennessee College of Medicine;* [d]*Research Service, Veterans Affairs Medical Center, Memphis, Tennessee, U.S.A.*

Accepted by Prof. V. Darley-Usmar

(Received 5 November 2000; In revised form 14 February 2001)

Hypotensive episodes during hemodialysis in patients with end-stage renal disease in the absence of inadequate maintenance of the plasma volume, preexistence of cardiovascular disease, or autonomic nervous system dysfunction is accompanied by increase in the plasma concentrations of the end-products of nitric oxide metabolism, above the levels expected based on the reduction of urea. Factors that can influence the synthesis of nitric oxide or the regulation of the effects of this free radical in patients with chronic renal failure are reviewed. Convergence of these factors and their interactions during the hemodialysis procedure are discussed as the basis for the generation of excessive amounts of nitric oxide that serves as an important contributing factor in the development of symptomatic hypotension.

Keywords: Nitric oxide/nitric oxide synthase, hemodialysis hypotension, asymmetric dimethylarginine, dimethylarginine dimethylamine hydrolase, von Bezold-Jarisch reflex

INTRODUCTION

During hemodialysis in the treatment of end-stage renal disease (ESRD), up to one-third of patients who do not suffer from chronic hypotension during the interdialytic period can experience severe hypotension, which can lead to the premature termination of the dialysis procedure and the incomplete removal of waste products.[1–3] Repetitive occurrence of these episodes can lead to diminution in both the quality and span of life.

The basis for the hypotension is multifactorial. Failure to preserve the plasma volume, preexistence of cardiovascular disease, or autonomic nervous system (ANS) dysfunction can all lead to hypotension during hemodialysis,[4–6] but

*Presented in part at the 10th Biennial International Conference of the Society for Free Radical Research held in Kyoto, Japan on October 18, 2000

†Corresponding author. Fax: 901-448-7265.

hypotension can also occur in their absence.[7,8] During investigations of variations in blood volume, stroke volume, cardiac output and systemic vascular resistance on a continuous basis during hemodialysis in a large cohort of patients utilizing bioelectrical impedance cardiography, the principle difference was a highly significant decrease in systemic vascular resistance due to inadequate compensatory response to the procedure in the 35% of patients who suffered symptomatic hypotension in the study.[9] In support of these findings, reanalysis of previously published data indicated that pooling in the microcirculation which contains nearly 40–50% of the total blood volume, not hypovolemia, was the likely factor causing hypotension during hemodialysis.[10] With calculated fluid volume replacements during dialysis apparently adequate, decrease in systemic vascular resistance would have to be explained by either excessive peripheral vascular dilatation, or movement of fluid from the intravascular to the interstitial space, or a combination of these two processes.

Nitric oxide (NO) is a highly reactive endogenously synthesized free radical which plays a major role in various physiological processes including the maintenance of vascular tone and the regulation of vascular permeability.[11,12] Metabolic derangements arising from the chronic renal failure and treatment by hemodialysis can modify the generation of NO in a number of ways, as listed in Table I. In this review, each of these factors and their interactions are discussed from the point of view of their likely impact on the generation of excessive amounts of NO that could lead to the development of symptomatic hypotension during hemodialysis.

TABLE I Factors regulating NO generation and NO signal transmission during hemodialysis in end-stage renal disease

A. Compatibility of agents used during hemodialysis
B. Presence of infection
C. Mechano-physical effects of flow and hypertension
D. Red blood cells and serum albumin
E. L-arginine availability
F. Reactive oxygen species
G. Accumulated endogenous NOS inhibitors
H. Enzymatic breakdown of NOS inhibitors
I. The von Bezold-Jarisch reflex
J. Other possible factors

Compatibility of Agents used during Hemodialysis

Hemodialysis brings immunologically responsive cells in contact with the dialysis fluid and with membranes of variable degrees of biocompatibility, which can eventually lead to the induction of an isoform of NO synthase with a high capacity for the synthesis of NO.[13]

Chemical Composition of Dialysate

The composition of the dialysate fluid has shown to be important in hemodialysis hypotension. Hypotensive episodes occurred more often with acetate than bicarbonate based dialysates. However, while replacement of acetate by bicarbonate decreased intradialytic hypotension, the total removal of acetate from the dialysate fails to entirely eliminate its occurrence.[14] Plasma collected from patients after acetate dialysis stimulates human umbilical vein endothelial cell NO synthesis more than the predialysis plasma or samples collected after bicarbonate dialysis or acetate-free biofiltration.[15] In addition, plasma interleukin-1β (IL-1β) concentrations are higher after use of acetate than other types of dialysates using the same types of membranes. These findings suggest that when acetate dialysates are used, NO and cytokines are released in excess which may contribute to hemodynamic instability during dialysis. However, other factors must be involved in hemodialysis related cardiovascular instability since the absence of acetate in dialysates does not eliminate these episodes.

Heparin

Heparin is used routinely during hemodialysis. Heparin increases circulating human hepatocyte growth factor (hGF), which induces endothelial

proliferation. Furthermore, the administration of hGF has been shown to cause NO-mediated hypotension in animal studies.[16] In a large cohort of patients undergoing maintenance hemodialysis, serum levels of hGF before dialysis were higher in a subset of patients with lower predialysis blood pressures (BP) and levels were higher after dialysis in the same group. Serum hGF levels correlated directly with plasma nitrate concentrations, suggestive of a role for this factor in hypotension by affecting endogenous NO production.[16] However, heparin is universally used in hemodialysis while hypotension occurs in only 30% of dialyses, thereby reducing the importance of heparin in hemodialysis hypotension.

Dialysate and Body Temperatures

In animals, cooling potentiates the endothelial response to cholinergic stimuli, increasing NO synthesis in cutaneous vessels, whereas in deep arteries, this response is inhibited.[17] The possibility that the removal of heat by the extracorporeal circuit together with or without the autoregulatory mechanisms attempting to preserve core temperature might underlie the hypotension was studied.[18–20] Cool dialysate reduced hypotension[21] as did the use of midodrine, an oral selective α-1 agonist in other studies,[22,23] whereas a combination of the two therapies did not provide additional benefit.[24] These findings suggest the autoregulatory mechanism to preserve core temperature may have a role in the development of hypotension in some instances.

A significant proportion of hemodialysis patients have subnormal body temperatures prior to dialysis. Therefore, whether the response to cool or normal temperature dialysate differed in patients with cool as opposed to normal temperature was investigated.[25] The incidence of symptomatic hypotension in euthermic patients was not affected by dialysate temperature, but hypothermic patients dialyzed against 37 °C dialysates had the highest incidence of symptomatic hypotension. In hypothermic patients, the incidence of hypotension decreased markedly upon dialysis with 35 °C dialysates. Thus, the hemodynamically protective effect of cool dialysates only occurs in patients with subnormal temperatures. The combined effect of the preexistence of subnormal body temperature and the use of cool dialysates during hemodialysis on the production of NO as measured by the plasma NO metabolites remains to be comprehensively examined.

Biocompatibility of Dialysis Membranes

The process of hemodialysis allows cells of the blood to be in contact with the dialyzing membrane resulting in varying degrees of immunological stimulation of responsive cells, depending on the biocompatibility of the membranes selected.[26] Activation of the complement cascade by contact of blood with bioincompatible membranes stimulates cytokine production, including IL-1β and tumor necrosis factor-α (TNF-α) which are increased in hemodialyzed patients.[26–29] The cytokines that are released following the initiation of the cascade induce NOS.[13] Moreover, exposure of mononuclear cells from previously dialyzed patients with low concentrations of lipopolysaccharide (LPS) produces five times more IL-1β than do cells from normal subjects.[30,31] Thus, mononuclear cells from dialyzed patients already contain IL-1β, which they continue to release even in the absence of exogenous stimuli in contrast to cells from undialyzed, healthy subjects.

Both IL-1β and TNF-α are powerful inducers of nitric oxide synthase or iNOS, which can occur within hours of exposure of responsive cells to these cytokines.[13] Among the three principal isoforms of NOS (NOS, EC 1.14.13.39) that have been identified, iNOS requires time before the catalytic conversion of L-arginine to NO and citrulline can occur whereas the constitutively expressed brain and endothelial forms, nNOS and eNOS, can produce NO without

delay.[12,32,33] Once induced, if the expression of iNOS were to extend into the next dialysis treatment, it could lead to the excessive production of NO. The possibility was raised that hypotension during maintenance hemodialysis in ESRD subjects could be the result of macrophage-dependent overproduction of NO in 1992.[34] Subsequently, NO synthetic ability was reported by us to be already expressed in the predialysis buffy coat cells from two-thirds of chronically dialyzed patients.[35] Moreover, buffy coat NOS activity appeared within the first hour of dialysis in several other patients where activity in the predialysis sample had not been detectable. These findings indicate that iNOS is likely to have been induced in the majority of hemodialyzed ESRD patients, but expression of activity may be masked until an inhibitor is removed.[35]

Amore *et al.* examined the effects of the hemodialysis membranes on the circulating cells from a healthy normal donor and the effects of such blood on iNOS of a murine endothelial cell line in culture.[36] NOS activity of the endothelial cells was stimulated by blood dialyzed with cuprophan, peaked at 15 minutes, rising 11-fold, whereas dialysis with the polymethylmethacrylate (PMMA) membrane was ineffective. Dialysis with cuprophan but not with PMMA induced the expression of mRNA encoding for iNOS in the endothelial cells. IL-1β and TNF-α were released after 6 hour by recirculating lymphocytes, paralleling the NOS activity profile in endothelial cells and was significantly higher after cuprophan exposure than PMMA.[36] Thus, the nature of the membrane has a significant impact on the induction of NOS. However, despite the use of membranes without the iNOS inducing effect, hypotensive episodes continue to occur during hemodialysis.

Earlier, Noris *et al.*[37] noted higher NO products in the plasma of dialysis treated uremics along with higher L-arginine concentrations than in controls. Platelets from uremics generated more NO and cyclic GMP (cGMP) than controls and these observations led them to ascribe the occurrence of hemodialysis hypotension to the predialysis presence of increased NO biosynthesis. However, in a study of patients with varying degrees of chronic renal failure each of whom was not yet placed on dialysis treatment, the urinary NO excretion was significantly lower in those with moderate to severe renal failure compared to normal controls and patients with mild renal failure.[38] Twenty-four hour urinary NO excretion of NO per mg creatinine correlated with renal function in all patients, directly with creatinine clearance, and inversely with the serum creatinine levels. Plasma concentrations of NO, which were higher in uremics than controls because of their diminished excretion, failed to correlate with the creatinine clearance of the serum levels of creatinine.[38] These findings indicated that patients with uremia actually produce less NO than controls. NO metabolites increased in the plasma because of their diminished excretion in the urine.

Chronic Hypotension in the Interdialytic Period

Interestingly, subsets of ESRD patients on maintenance hemodialysis exhibit chronic hypotension in the interdialytic period. In a study of such patients with predialysis systolic BPs < 100 mm Hg, higher plasma nitrite and nitrate (NO_x) levels were reported than in normotensive patients and the mean arterial pressures (MAP) of both hypotensive and normotensive patients correlated inversely with the plasma NO_x.[39] The adjusted mortality risk is significantly associated with low predialysis BPs in a national sampling of maintenance hemodialysis patients. Patients with BPs < 110 mm Hg had an elevated relative mortality risk compared to those with higher predialysis BPs ($p < .0001$), whereas no association with an elevated mortality risk was observed for predialysis systolic hypertensives except for an elevated risk of cerebrovascular deaths.[40] In this review, the possible cause(s) of chronic hypotension during the interdialytic period are not

considered. The focus of this review concerns hypotension occurring only during the process of hemodialysis in ESRD patients on long-term maintenance dialysis.

Presence of Infection

Hemodialyzed patients experience a high rate of infection with various microorganisms that also leads to the synthesis of cytokines with effects on upregulating NOS.[13] The higher rate of interdialytic infection than controls of comparable age[41,42] occurs irrespective of the presence or not of diabetes.[43] Dialysis access infections and pneumonia are the two most common infections seen in hospitalized patients receiving dialysis treatment[44] and the variety of infectious complications in ambulatory hemodialyzed patients is also high and well documented.[45] Unintentional pathogens can be introduced during hemodialysis by means of the access with gram positive organisms most commonly involved and Staphylococcus aureus, coagulase negative predominating.[46] Clotted nonfunctioning grafts are harbingers of infection and are likely sources of infection even in the absence of clinical signs of graft site infection.[47] Approximately 20–30% of this population is infected with the Hepatitis C virus,[48] a virus also recognized as a cause of membranoproliferative and membranous glomerulonephritis that can be the basis for the renal failure. Hepatitis B viral transmission through blood transfusions given for severe anemia and through environmental transmission in the dialysis setting can also occur in hemodialysis patients.[49] In the United States, infection is a major cause of mortality in the dialysis population, accounting for 12% of all deaths among the hemodialyzed with septicemia occurring in 76% of those who died.[50] Older age and lower serum albumins are independent risk factors for septicemia in hemodialyzed patients.[43] In a longitudinal (seven year) cohort study of the U.S. Renal Data System, temporary vascular access and dialyzer reuse, a common practice, were also associated with increased risk for septicemia.[42]

Other risk factors that contribute to higher rates of infections amongst these patients include a number of immunological deficits found in uremics, such as impaired lymphocyte mitogenic response and granulocyte abnormalities with impaired phagocytosis, respiratory burst and myeloperoxidase activities, as recently summarized.[51] Impaired functions of the immunological cells are ascribed to a range of abnormalities from diminution in the expression of surface adhesion molecules[52] with release of intracellular adhesion molecule-1, vascular cell adhesion molecule-1,[53] and leukocyte surface L-selectin (CD 62L) into the plasma,[54] to enhanced DNA fragmentation of monocytes upon *in vitro* culture.[55] In addition, anemia developing in the majority of ESRD patients is managed by recombinant human erythropoietin (rHepo) and intravenous iron supplementation with positive effects on the anemia. However, iron is also an essential nutrient for microorganisms raising concerns about a potential link between excessive iron supplementation and the risk of infection in patients with ESRD.[56]

Infection contributes further to the release of cytokines such as IL-1β, TNF-α and others, raising the levels already enhanced by activation of the immunologically responsive cells to the dialysate and membrane used in hemodialysis with significant effects on the induction of NOS and the generation of both NO and other reactive oxygen species (ROS). However, although interim infection can induce NOS in various tissues and the immunologically responsive cells circulating in the blood, not all patients with infections experience hypotension and hypotension can also occur in the absence of infections.

Mechano-physical Effects of Flow and Hypertension

The mechano-physical effects of the dialysis process could alter the generation of NO by the

resistance vessels, particularly in hypertensives, where vasoconstricted vessels would be expected to be under higher shear stress, enhancing the synthesis of NO by the vascular endothelial cells,[57] thereby affecting various intravascular functions of NO.

Vascular Tone and Shear Force

In the absence of an infection, NO is unlikely to be formed in significant amounts except by the vascular endothelial cells. Increments in vascular flow induce proportional increases in vascular diameter which are abolished by an antagonist of NO synthesis.[58] The elevation of intracellular cGMP upon stimulation of soluble guanylate cyclase by NO is proportional to the intensity of the shear stress exerted in experimental systems.[59] The release of NO by vascular tissue increases six-fold within 1 minute upon increasing flow eight-fold.[60] Shear stress, initiated by flow, causes basal release of NO *continuously in vivo*.[61] The expression of eNOS via a transcriptional mechanism has been demonstrated where unidirectional shear stress dramatically increases eNOS mRNA.[62] Thus, shear stress exerted on the vascular walls by the streaming blood is the most important stimulus for NO release by endothelial cells, by up-regulating constitutive eNOS.[62,63]

The basal release of NO by the vascular endothelium is essential to the maintenance of a constant vasodilator tone. With advancing age there is diminution in the production of NO on a generalized basis, as evidenced by a reduction in urinary nitrate excretion and diminution in NO-mediated vasodilatation in the forearm vascular bed.[64] Vascular reactivity to noradrenaline is also blunted in healthy older people,[64] and the generally older age of those with hemodialysis hypotension[35] suggests that blunting due to natural aging could be another factor contributing to hemodialysis hypotension.

It is likely that the enhancement of shear would be greater in hypertensives than in normotensives. Patients with chronic renal failure are often hypertensive and develop anemia sometime during their long sojourn to end-stage for which rHepo has proven useful, but for the development of hypertension or the aggravation of pre-existent hypertension in many.[65] In adult rats, the hypertension resulting from chronic inhibition of NO synthesis using N-ω-nitro-L-arginine methyl ester or L-NAME significantly changes the myocardium and the blood vessels, with hypertrophy of the myocytes and fibrosis, and diminution in the absolute volumes of the blood vessels.[66] These changes are not due to the arterial hypertension *per se* as prevention of L-NAME hypertension with hydralazine does not affect the development of the microvascular remodeling and cardiac hypertrophy.[67] However, the hypertensive response to L-NAME can be greatly attenuated by sympathectomy performed either before treatment with L-NAME or after the development of the hypertension,[68] raising the importance of the nNOS over eNOS in this regard. In blood flow studies of the normal human skin and digits, NO appears to contribute to basal vasodilator tone, but not during reflex sympathetic vasoconstriction.[69] Thus, a complex relationship appears to exist between the signals transmitted by the sympathetic reflex and those arising from NO generated by the vascular endothelial cells during normal blood flow.

Vascular Permeability and Cellular Adhesion

Inhibition of NO synthesis with L-NAME produces a five-fold increase in microvascular fluid and protein flux, which can be reversed by nitroprusside.[70] Leukocyte adhesion is profoundly increased by inhibition of NO synthesis and is reversible only in part by nitroprusside.[70] Adhesion of leukocytes to the microvascular wall is tightly coupled to emigration of these cells and leakage of fluid and proteins following ischemia.[71] These effects are primarily mediated by the adhesion glycoproteins

CD11b/CD18 on activated neutrophils and the intercellular adhesion molecule-1 (ICAM-1) on the vascular endothelium and appear to require L-selectin-dependent leukocyte rolling.[71] NO and the prostaglandin prostacyclin are involved in the bidirectional regulation of basal microvascular permeability,[72] accounting for 30–40% change in fluid movement in either direction above basal values. These effects are additive for these factors, and whereas NO is involved in the regulation of basal vascular tone in a major way, prostacyclin is not.[72]

Vascular endothelial growth factor (VEGF), the endothelium secreted protein, induces vasodilatation, increases the release of NO by the endothelium, and is a potent inhibitor of leukocyte-endothelium interaction, which correlates with the ability of VEGF to augment the release of NO.[73] Interestingly, heparin used extensively during hemodialysis (see above) exerts anti-inflammatory effects in addition to its anticoagulant properties. In a model of inflammation using TNF-α in the rat, where significantly increased leucocyte rolling, adhesion, and migration and vascular permeability were seen, heparin pretreatment significantly attenuated cellular rolling, adhesion, and migration but did not affect expression of cell adhesion molecules or vascular permeability.[74]

Thus, normally, prevention of movement of fluid and proteins across blood vessels depends on the synthesis of a basal amount of NO by the endothelial cells which would also prevent leukocyte adherence. On the other hand, excessive generation of NO can also lead to increased vascular permeability and migration of inflammatory cells as seen following allergen challenge,[32] indicative that a balance between too little and too much NO and other mediators must be maintained for vascular permeability to be normal.

Hydraulic Turbulence

Anemia is often present in these patients many of whom are treated successfully with rHepo.[65] In the recovery phase during treatment with rHepo, cells of variable sizes are often likely to be present including normal sized red blood cells (rbcs), senescent cells (smaller), oxidatively damaged cells, and reticulocytes (larger). During normal blood flow, the central core of the rapidly moving blood would contain cells of fairly uniform size in rouleaux formation with smaller cells and platelets in the periphery and a cell-free layer adjacent to the endothelial surfaces of the vessels (see Figure 1, panel (b)).[75,76] Hydrodynamically, like fine particles, smaller cells would be expected to create greater turbulence than the larger cells in the central core and interaction with the vessel wall could occur in a random manner creating chaotic motion and turbulence. Together with the ability of these cells to bind NO through the hemoglobin they contain, a gradient for the gaseous NO produced by the endothelial cells might be produced which may be far greater than if all the cells were of uniform size. Such a gradient would likely siphon more NO towards the luminal surface of the vessels, reducing the amount that would diffuse toward the vascular smooth muscle cells (VSMC) and interfering with the ability of the vessels to maintain normal vascular tone (see Figure 1, panel (d) compared to (c)). The development of hypertension following rHepo treatment occurs several months after initiation of therapy, not immediately,[65] a time when the circulating cells would likely be of variable sizes. In a large cohort of rHepo treated patients seen in our clinics, an inverse correlation between the cell size or mean cell volume (MCV) and the predialysis systolic BP can be seen (see Figure 2). A direct correlation between the predialysis systolic BPs and the degree of hypotension during hemodialysis had been reported earlier for these rHepo treated patients.[35,77]

Red Blood Cells and Serum Albumin

Hypoproteinemia and anemia are often found in chronic renal failure. NO can reversibly bind to

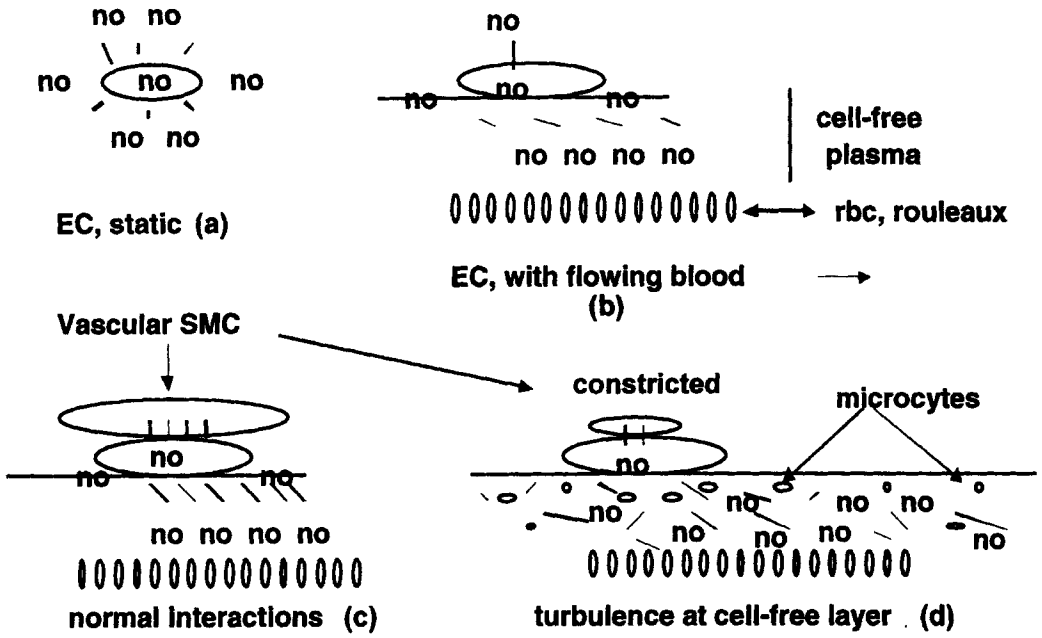

FIGURE 1 Rheology, NO and RBC MCV. Figures in four quadrants: (a) endothelial cells (EC) without flow (EC, static); (b) EC, with flowing blood showing cell-free layer of plasma adjacent to EC of the artery and rbcs of uniform size in rouleaux formation; (c) normal interactions in hemisection of an artery with rbcs in rouleaux, not creating turbulence, maintaining normal vascular smooth muscle cell (SMC) contraction; and (d) hemisection of artery under flow in presence of microcytes creating a gradient of NO through turbulence, reducing amount of NO reaching the vascular SMC causing the SMC to contract (turbulence at cell-free layer).

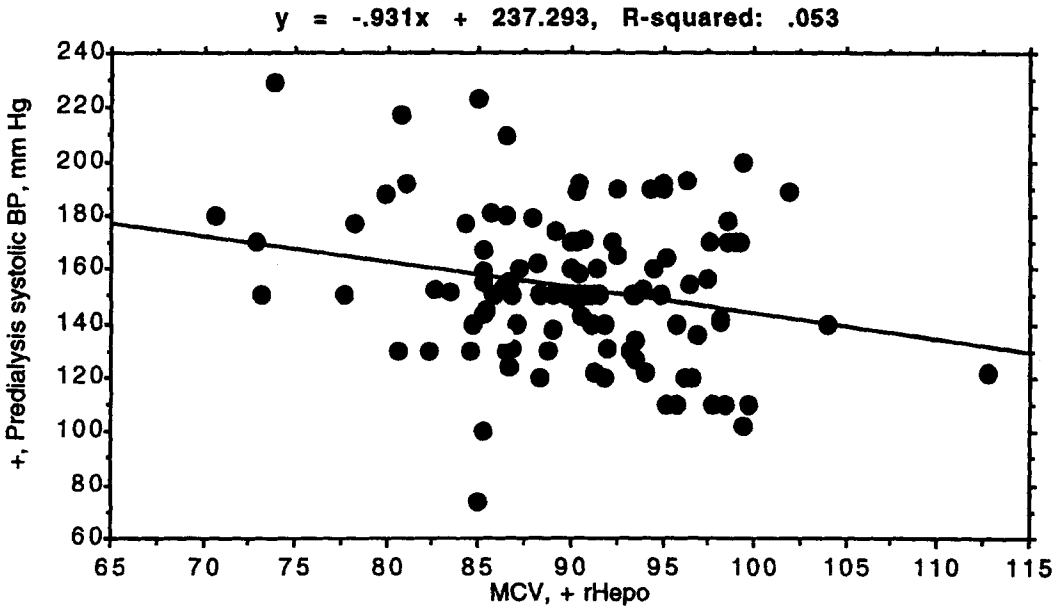

FIGURE 2 Correlation between the predialysis systolic BP and the rbc mean corpuscular volume in rHepo treated hemodialyzed ESRD patients. $p = .02$.

hemoglobin and albumin under physiological conditions.[78–80] More often than not, the anemia requires the administration of rHepo for treatment. The use of rHepo is associated with the development of hypertension in many patients with uremia,[65] further complicating hemodynamic regulation during dialysis. Additionally, iron overload resulting from overzealous treatment of the anemia could also interfere with NO effects, not only from its direct interaction with NO but, perhaps, through the ability of iron to support the growth of infectious agents.

NO synthesized by the vascular endothelial cells would be released both luminally and abluminally, being a gas (Figure 1, panel (c)). Abluminally, the NO released would enter the surrounding smooth muscle cells and activate soluble guanylyl cyclase transmitting the signal to dilate.[81] NO released by the vascular endothelial cells into the luminal space might be expected to be entirely scavenged by the hemoglobin in the rbcs, as the molar concentration of hemoglobin far exceeds the amount of NO that could be released by the endothelial cells. During flow, a cellular-free zone of plasma forms adjacent to the endothelial lining of the vessels with a core of rbcs (Figure 1, panel (b)).[75,76] The cell-free zone allows the NO released by the endothelial cells to interact with dissolved oxygen for conversion to nitrite as well as other soluble components in the plasma. The remaining NO would reach the red cell layer, interacting with the proteins and sulfhydryl compounds within these cells. In severely anemic patients with chronic renal failure who are treated with rHepo, hypertension can develop or pre-existent hypertension can be aggravated, whereas anemic patients with normal renal function do not experience this side effect.[82,83]

NO interacts with the iron moieties of proteins and the reversibility of this process underlies its effectiveness in the regulation of various physiological processes.[78,79] NO can also avidly interact with the cysteine or sulfhydryl constituents of proteins including albumin[78] and the hemoglobin in the rbcs.[79] Both the iron of hemoglobin and the cysteine in the globin chain of hemoglobin, βCys93, can interact with NO, binding NO or releasing NO depending on the state of oxygenation and the pH of the plasma.[80] S-nitrosylation of βCys93 of albumin allows this protein to serve as an endogenous NO donor and a physiological regulator of BP and tissue perfusion, depending on the presence of low-molecular-weight thiols.[84] Normal amounts of serum albumin and hemoglobin concentrations could offset an excessive formation of NO. Since hypoalbuminemia and anemia are often present to varying degrees in ESRD, the pool of NO "binders" in the blood could play a role in the development of hypotension during hemodialysis.

Roccatello *et al.*[85] measured nitrosylhemoglobin during hemodialysis detected by electron paramagnetic resonance over the duration of a standard bicarbonate dialysis, comparing patients dialyzed with different membranes. Basal levels of nitrosylhemoglobin were higher in ESRD subjects than controls and were similar for peritoneal and hemodialysis groups. Within 15 minutes of hemodialysis, a significant increase in nitrosylhemoglobin was seen irrespective of the type of membrane used. No change in the blood nitrosylhemoglobin was seen in peritoneally dialyzed patients.[85] In our own study using a biochemical approach, this early increase in the rbc nitrosylhemoglobin concentration was corroborated in those with symptomatic hypotension as opposed to those with normotension where nitrosylhemoglobin reduction occurred.[86] These findings indicate that hemoglobin in the rbcs can sequester NO during periods of excessive formation coinciding with the development of symptomatic hypotension, but hypotension during hemodialysis is not the result of the release of NO from hemoglobin. Instead, sequestration of NO by hemoglobin more likely modulated the degree of hypotension that occurred in these patients.

L-Arginine Availability

Since the kidney is the major site of synthesis of L-arginine,[87,88] the substrate for NOS, preservation of renal arginine synthetic capacity might be expected to differ among patients depending on the underlying cause and extent of the renal damage. L-arginine can be synthesized by the hepatic urea cycle, but the arginine produced by the liver is not readily available for utilization by the body. High levels of arginase prevent most of the liver-synthesized as well as dietary arginine to reach the peripheral circulation, instead regenerating L-ornithine for the continued disposal of ammonia as urea.[89] Furthermore, much of the ingested arginine is converted to L-citrulline by enzymes in the intestinal walls.[89] The citrulline is then transported to the kidneys for enzymatic conversion to L-arginine and release into the blood[88] for utilization in protein synthesis, NO synthesis, and other enzymatic reactions.

In the kidney, arginine is produced almost exclusively in the proximal tubule, with decreasing intensity from the early convoluted part to the medullary straight part.[90] In rats with 5/6 nephrectomy with significant reduction in renal plasma flow and glomerular filtration rate, maintenance of normal arginine synthesis was seen most likely from the cumulative effects of hypertrophy of the remnant proximal convoluted tubules, hyperfiltration in remnant nephrons, and high plasma levels of L-citrulline.[91,92] With severe renal failure in rats, however, there is minimal hypertrophy in the remnant kidney and significant reduction of arginine synthetic capacity.[93] Thus, a critical level of renal tissue/function must be retained for hypertrophy of the renal tissue to occur.

An increase in the plasma citrulline is one of the earliest and most frequently reported finding in humans, rats, and dogs with chronic renal failure.[94,95] For plasma arginine, normal,[96,97] increased,[37] and low[98] levels have been reported. In a small group of ESRD patients who were stable, without intercurrent illness, and who were on an ideal regimen of three 3-hour sessions of hemodialysis per week providing 65% urea clearance, isotope studies revealed that endogenous arginine synthesis was within the normal range with 45–65% of the net rate of arginine synthesized used in NO synthesis compared to 12% in controls.[97] Importantly, using tracer amino acids, *citrulline* fluxes were 4–5 times higher in ESRD patients than in healthy controls reflective of the ability of these patients to convert citrulline to a reusable substrate at an elevated rate, most likely through its conversion to L-arginine.[97]

Variability in plasma arginine levels may reflect differences in residual arginine synthetic capacity of the kidneys or diminished intake of protein. Malnutrition and decrease in lean body mass are often seen in these patients[94,99] and protein restriction used to delay renal function loss in these patients[100] could continue to be practiced. Anorexia is a frequent complaint and there is experimental evidence that dialyzable uremic toxins suppress appetite.[101] Other factors that contribute to malnutrition include the cytokines that are increased in the plasma enhancing protein catabolism and increasing protein requirements and the dialytic loss of amino acids and glucose.

Thus, depending on the underlying pathological process leading to renal failure, residual arginine synthetic ability and dietary intake of arginine could differ in these patients accounting, in part, for variability in substrate availability for the synthesis of NO by all isoforms of NOS.

Reactive Oxygen Species

Other ROS that interact with NO are also produced as a result of laminar shear stress[102] and infections, adding to the enhancement of ROS found in chronic renal failure[103] and serving to reduce, augment, or alter the signals transmitted by NO during hemodialysis. Defenses

against the production of free radicals and ROS are reflected by the plasma ascorbate, urate, α-tocopherol and in the rbc enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-x). In chronic renal failure[104] and hemodialyzed ESRD patients,[105] antioxidant defense mechanisms appear to be overtaxed as reflected by lower rbc SOD, GSH-x and CAT enzymes in these patients compared to controls. Derangements in antioxidant status could arise from the increased generation of ROS, as indicated by the higher spontaneous production of ROS in lymphocytes from patients with ESRD than controls,[106] as well as from the loss of antioxidant reserve. There is laboratory evidence of enhanced peroxynitrite formation in chronic renal failure patients. Co-generation of NO and superoxide yields peroxynitrite, which preferentially nitrates tyrosine residues of protein and non-protein origins.[107] Whereas nitrotyrosine is undetectable in plasma from normal volunteers,[103] it is present in chronic renal failure and rises four-fold during septic shock in these patients.

Diminished antioxidative capacity resulting from a deficiency of selenium has been reported which responds to parenteral supplementation with sodium selenite.[108] Other parameters indicative of lipoperoxidation of rbcs are also seen in chronic renal failure patients with elevated levels of free malondialdehyde (MDA) and diminished levels of polyunsaturated fatty acids (PUFA), particularly arachidonic acid, prior to placement on a reduced dietary protein regimen, which corrects these derangements to some degree.[109] Comparison of six different membranes on oxidative stress was done by measuring plasma homocysteine, cysteine, MDA, GSH, glucose-6-phosphodehydrogenase, glutathione reductase, GSH-x, CAT, and SOD activities. Wide variability in changes reflecting oxidative stress was found in hemodialyzed patients, which were only in part due to differences in the membranes used.[110] Increase in infectious complications would also contribute to increasing the generation of ROS and diminution of antioxidant defenses.

Oxidative injury to rbcs contributes to shortening their half-life.[82] Treatment with rHepo significantly improves rbc volume, the hemoglobin concentration, the hematocrit and the reticulocyte count. However, rbc oxidative sensitivity and deformability, the splenic rbc volume and the intrasplenic transit time are not improved, indicative that extra-rbc factor(s) are also contributory to reducing the rbc life span.[111]

Vaziri *et al.* demonstrated that increased ROS activity inactivated not only NO, but oxidized arachidonic acid and exerted a direct vasoconstrictive action in rats, which could be blocked by the intravenous administration of an hydroxyl radical scavenger, but not by SOD or CAT.[112] These findings indicate that hydroxyl radicals may be the major ROS produced that inactivates NO resulting in the hypertension found in uremic rats.[112] Increases in blood flow trigger free radical release *in vivo* and in isolated perfused rabbit aortae.[113] In particular, H202 is produced by cultured porcine aortic endothelial cells subjected to cyclic strain mechanical deformation.[114] Intracellular superoxide production in human umbilical vein endothelial cells is elevated within minutes from the onset of laminar sheer stress and is maintained at the elevated level as long as flow continued for a 6 hour period of time.[102]

Enzymes that can supply free radicals other than NO include xanthine oxidase, cytochrome P-450, cyclooxygenase, lipoxygenase, and the superoxide-generating NADPH oxidase. Recently, shear-induced ROS production has been shown to mediate the tyrosine phosphorylation and, presumably, activation of the mitogen-activated protein kinase, perhaps one of the kinases that phosphorylates eNOS leading to the sustained release of NO.[115] Phosphorylation into two specific eNOS tryptic peptides as early as 30 seconds after initiation of flow has been reported.[116] These finding suggests that endothelial cell free radical production may exert

an autocrine role in the control of vascular tone by shear stress.

Activation of NOS with suboptimal concentrations of L-arginine or the cofactor, tetrahydrobiopterin leads to the synthesis of ROS, including peroxynitrite.[117] The parenteral administration of peroxynitrite to rats produces profound hypotension followed later by hypertension.[117] Considering the state of malnutrition in these patients and that dialysis of the blood is repeated 2–3 times a week, limiting concentrations of these factors are likely to be present in some patients, perhaps contributing to the excessive formation of ROS, including peroxynitrite.

Accumulated Inhibitors of NO Synthesis

Whatever the underlying etiology leading to end-stage, the kidneys fail to excrete metabolic end-products leading to the accumulation of several compounds or "renal toxins" with clinical consequences. Among the accumulated metabolites that fail to be excreted in ESRD are methylated L-arginine derivatives such as asymmetric dimethylarginine (ADMA) and NG-monomethyl-L-arginine (LNMMA), which are competitive inhibitors of NOS. ADMA is post-translationally synthesized on arginine residues of non-histone proteins located in the nuclei of cells by a specific enzyme, protein methylase I (E.C. 2.1.1.23). After release during nuclear protein turnover, ADMA is not utilized for incorporation into protein[118–121] and the renal threshold for the methylated arginines is low.[122] Consequently, increase in their concentrations would be expected in chronic renal failure, as reported.[35,123–125] The concentrations of one of these inhibitors, ADMA, is highly variable among hemodialyzed ESRD patients.[35,125] Since they are dialyzable,[35,124] concentrations at the end of dialysis might be expected to be the starting point for the further accumulation of this inhibitor during the interdialytic period. In addition, strong evidence for the metabolism of ADMA has appeared, as discussed below.

We reported that the higher the ADMA levels, the higher the predialysis systolic BP and the greater the reduction in BP during dialysis.[35] The plasma ADMA also correlated directly with the predialysis pulse. However, levels of plasma NO_x did not correlate with the plasma ADMA. ADMA levels were higher in females, older patients, diabetics, patients with interim infections. Patients on erythropoietin treatment and older patients and females suffered more severe hypotension in our study.[35] These findings indicated that the level of the NOS inhibitor, ADMA, could be a contributing factor in the hypotension during hemodialysis.

Others also noted markedly different elevations of plasma ADMA in hemodialyzed ESRD subjects.[125] The increase was six-fold compared to controls and to peritoneally dialyzed ESRD patient. ADMA levels were higher in patients with manifest atherosclerotic disease than in those without this risk factor for endothelial and cardiovascular dysfunction. Together with the higher incidence of symptomatic hypotension reported in elderly patients, the majority with long-standing histories of hypertension and atherosclerosis,[126] a role for ADMA in the symptomatic hypotension during hemodialysis is rather compelling.

During hemodialysis, the concentrations of ADMA and LNMMA are reduced, allowing NO synthesis to be resumed by all of the isoforms of NOS, provided substrate is available. Active transport of L-arginine into cells enables intracellular L-arginine concentrations to be higher than the extracellular fluid, but methylated analogs of L-arginine such as LNMMA can inhibit the transport of L-arginine.[127] In addition, "trans" stimulation of the uptake of L-arginine in VSMC can occur where cells preloaded with LNMMA increase the uptake of L-arginine.[128] Assuming "trans" stimulation was effective in these patients, more arginine would likely be taken up when the plasma and hence the intracellular concentrations of these methylated arginines are higher. Since the rate

of NO synthesis is a function of substrate concentration, higher intracellular arginine concentrations arising from the "trans" stimulation of L-arginine transport into cells would better compete with the methylated arginine inhibitors of NOS, perhaps increasing the rate of NO synthesis, assuming cofactors are sufficiently available. Thus, the high variability of the plasma concentrations of ADMA found in hemodialyzed ESRD patients[35,125] could evoke in some patients the "trans" stimulation of L-arginine to produce more NO by endothelial cells in patients with higher ADMA levels than where ADMA levels are lower. This could explain the correlation found between ADMA levels and the drop in BP reported earlier by us.[35]

Evidence that endogenously accumulated inhibitors modulate NOS activity during the process of dialysis is highlighted by failure to detect NOS activity in the predialysis samples from one-third of the patients and its appearance in many as dialysis progressed.[35] Predialysis NOS activities did not differ based on blood pressure reductions. However, patients with higher predialysis systolic BPs, had greater increases in NOS activity after the first hour of dialysis, as though an inhibitor had been removed. And change in NOS activity after the first hour correlated directly with the percent drop in BP that occurred.[35] It might be argued that recruitment of immunoregulatory cells to the vascular compartment from other sites or from adherence to the vascular walls[129,130] accounted for the increase in NOS activity. However, such cells would have been influenced by the same stimuli that induced NOS and other factors that inhibited NOS activity. The resumption of iNOS activity occurring after 1 hour of dialysis would likely also result in the resumption of activity by other isoforms of NOS, including the enzymes in endothelial cells lining the vasculature.

Increases in plasma ADMA levels have also been reported together with diminution in NO metabolites in a wide variety of diseases that are found in ESRD, including hypertension in both adults[131] and children,[132] peripheral arterial occlusive disease,[133] congestive heart failure,[134] schizophrenia,[135] hypercholesterolemia[136] and arteriosclerosis. It could be that the presence of these disorders in the patient with ESRD further elevates the levels of the methylated inhibitors of NOS, enhancing the vulnerability of patients to cardiovascular instability during hemodialysis.

Enzymatic Degradation of L-Methylated Inhibitors of NOS

Some of the endogenous methylated arginine analogs that serve as inhibitors of NOS can be enzymatically converted to L-citrulline and mono- or dimethylamines.[122] The status of this enzymatic activity would greatly influence the levels of the NOS inhibitors. The enzyme, dimethylarginine dimethylaminohydrolase or DDAH, hydrolyzes ADMA to L-citrulline and dimethylamine, a precursor of the powerful carcinogen, dimethylnitrosamine. LNMMA is also hydrolyzed by DDAH to L-citrulline and monomethylamine. No cofactors are required and the enzyme is widely distributed.[137] Monoclonal antibodies have been produced[138,139] and recently, two isoforms have been identified.[140] The wide variability in the plasma levels of ADMA could be related to differences in the activity of DDAH in the various tissues in which it has been reported to be present.

DDAH is present in the circulating white blood cells, but whether it is expressed in the rbc had not been reported. To identify a tissue with ready accessibility for testing, we examined rbcs for DDAH activity. Recovery of L-citrulline, the product used to measure this enzyme in normal human rbcs, increased as a function of supernatant protein, incubation time and substrate concentration. SDMA did not serve as a substrate. Activities varied widely but mean values were not different between hemodialyzed ESRD patients and controls. Addition of the patients' plasmas to control enzymes increased

or decreased enzyme activity while normal plasmas had little effect on the rbc enzymes of ESRD patients. Increase in activity could be due to high endogenous levels of ADMA in the plasma raising the effective substrate concentration and diminution of activity must be due to the presence of DDAH inhibitors. The enzyme was inhibited by L-citrulline and by Fe, Mg, and $ZnCl_2$, but not by $CaCl_2$ or norvaline (manuscript under review and abstract to be presented, AFMR, New Orleans, LA, March 2, 2001). Considering the use of high amounts of iron in the management of anemia in these patients, the effect of iron on enzyme activity could have clinical relevance by diminishing the metabolism of ADMA and LNMMA, raising their intracellular and plasma concentrations. Exposure to NO for 1 minute reduced activity slightly suggestive that inactivation of the enzyme by NO synthesized by eNOS would have some role in the regulation of this enzyme. Rbc DDAH activity correlated inversely with age which is interesting considering that older patients tended to have higher ADMA levels. Using a monoclonal antibody to human DDAH, the presence of a 32 kDa protein was seen in cells from controls as well as patients. There was wide variability in the amount of protein observed which did not parallel enzymatic activity. Whether this was because of modification of the protein at antibody recognition sites, but not the catalytic site, or vice versa, has not been determined.

Undoubtedly, the activity of DDAH within the rbc as well as in other tissue cells would affect the concentrations of the NOS inhibitors that serve as substrates for DDAH, thereby affecting NO regulation. Analysis of other tissue forms of DDAH in ESRD have not been reported to date.

The von Bezold-Jarisch Reflex

While autonomic nervous system (ANS) dysfunction is present in many ESRD patients,[6] not all of those with ANS dysfunction exhibit hemodynamic instability, and hypotension can develop in others with apparently normal ANS function during hemodialysis.[6,141] In assessing the role of the ANS in dialysis hypotension, autonomic function tests have been used at rest, static exercise tests (efferent sympathetic function) and heart rate variability as well as Valsalva maneuver and deep breathing (parasympathetic function) at successive stages of a standard hemodialysis session in patients, which did not distinguish hypotension-prone from normotensive patients in some studies,[142,143] while sympathetic activation appeared to be impaired late in dialysis in the hypotension-prone in another study.[144]

Standard hemodialysis activates a marked and reversible sympathetic response in both hypotensive-prone and resistant patients. The primary hemodynamic perturbation induced by ultrafiltration is a decrease in atrial pressure resulting in a fall in cardiac output resulting in activation of sympathetic mechanisms that maintain systemic resistance and arterial pressure. Sustained hypotension in ESRD patients is characterized not only by overactivation of the sympathetic and renin-angiotensin systems but also by decreased vascular resistance and a blunted vascular response to pressor stimuli.[145] Cases *et al.* report that adrenomedullin levels are higher in hypotensive than in normotensive and hypertensive hemodialyzed ESRD patients and that the plasma levels of this peptide in hypotensive patients correlated inversely with mean arterial pressures during hemodialysis.[145] To Ligtenberg and his coworkers, sudden dialysis-related hypotension characterized by paradoxical vasodilatation was suggestive of sympathoinhibition that is evoked by lower body negative pressure when volume is reduced.[146] During dialysis, decrease in plasma osmolarity caused mainly by urea removal could contribute to reduction in vascular refilling.[147,148]

Intradialytic hypotension is often accompanied by paradoxical bradycardia.[149] The sequential changes in ANS activity up to and

during the hypotensive episode were monitored with continuous, beat-to-beat measurements of BP and heart rate during hemodialysis in hypotension prone and not prone patients. In patients without symptomatic hypotension, mean arterial BP hardly fell, whereas heart rate increased significantly. There was evidence of compensatory baroreflex-mediated activation of the sympathetic nervous system with suppressed parasympathetic activity during ultrafiltration-induced intravascular volume depletion. In patients with severe symptomatic hypotension, changes in heart rate and spectral analysis of heart rate variability were similar to those in patients without hypotension up to the moment of the development of symptoms (nausea, vomiting, dizziness, muscle cramps) of hypotension although mean arterial BP had only gradually dropped very minimally. There followed a sudden further reduction in the mean arterial BP accompanied by bradycardia.[149] Findings agree with activation of the cardiodepressor reflex involving decreased sympathetic and increased parasympathetic nervous system activity, representing a physiologic response (the von Bezold-Jarisch reflex) that is seen upon reduction of intravascular volume and reduced cardiac filling as the basis for the sudden intradialytic hypotension. Normally, reduction of blood volume causes sympatho-excitation leading to vasoconstriction and tachycardia. Initiation of the von Bezold-Jarish reflex leading to paradoxical vasodilation and bradycardia can follow fluctuations in vasomotor tone under hypovolemic conditions as this reflex serves as a safety valve, slowing the heart rate, increasing diastolic filling and decreasing after load.[150] In rats made hypertensive by administration of L-NAME to inhibit the synthesis of NO, the von Bezold-Jarisch reflex is exaggerated.[151,152] Enhancement of this reflex seemed to be, to a large extent, due to hyperresponsiveness of the cardiac pacemaker to cholinergic stimulation.[152]

To assess the prevalence of bradycardic hypotension, Zoccali *et al.* investigated 60 hypotensive episodes in 20 patients.[153] Tachycardia was the more frequent heart rate response to dialysis hypotension while bradycardia was associated with a hemodynamic profile indicating a more severe degree of cardiovascular underfilling.[153] In this regard, there is a strong association between left ventricular hypertrophy (LVH) and dialysis hypotension. LVH is common in ESRD, increases with time on dialysis, is present in up to two-thirds of patients on maintenance hemodialysis, and is associated with impaired diastolic relaxation.[154] By dobutamine-atropine stress echocardiography, hypotension-prone patients exhibit impaired myocardial contractile reserve compared to hypotension-resistant patients.[155]

Hand and coworkers studied *in vivo* responses of norepinephrine preconstricted dorsal hand veins to locally active doses of acetylcholine (stimulates NOS) and glyceryl trinitrate (a NO donor) in patients undergoing maintenance hemodialysis and in age and sex matched controls. Vasodilation in response to acetylcholine is impaired in hemodialyzed ESRD patients which was corrected by dialysis and co-infusion of L-arginine but not by D-arginine, while vascular response to glyceryl trinitrate was similar before and after dialysis. These findings strongly suggest that inhibitors of NOS play a role in endothelial dysfunction during hemodialysis in ESRD which are cleared by dialysis.[156]

Other Possible Factors

Reports had appeared that the human rbc contained NOS proteins[157–159] and that the enzymes were active.[157,158] If this were true, hemoglobin sites for NO would always be occupied, diminishing the role of hemoglobin in NO regulation. It is possible that the findings could as well have been explained by the release of NO from the many adducts present in rbcs where change in NO products was measured[157] in one report. In the other, arginase had not been inhibited during analysis of NOS activity.[158]

When rbc supernatants were incubated with labeled L-arginine plus all the required cofactors for NOS plus norvaline to inhibit arginase,[160] labeled product was not recovered.[161] Thus, NOS activity is not expressed by circulating human rbcs from control subjects. These findings indicate that hypotension during dialysis cannot be ascribed to the excessive formation of NO by the circulating rbcs.

Curiously however, our findings corroborate that rbcs do, indeed, contain the proteins of two isoforms of NOS, iNOS and eNOS, as published earlier.[161] It seems that rbcs might have expressed NOS activity sometime during ontogeny. Once released into the circulation, however, the NOS enzymes would not remain active for long as they contain iron and cysteines at their active and cofactor binding sites, the nitrosylation of which would inactivate them, in due course. The question could be raised as to whether younger cells that could be introduced into the circulation secondary to the effects of rHepo on erythropoiesis could express active NOS isoforms.

DISCUSSION

Increased NO synthesis or generation during hemodialysis as the basis for the symptomatic hypotension that occurs was initially suggested by Bealsey and Brenner[34] and first shown by Yokokawa *et al.*[8] In the latter report, a rise in the postdialysis plasma NO_x over the predialysis value was observed in patients with hypotension, whereas levels dropped in others. In one of our studies, more than half of the patients dropped their plasma NO_x in accord with their urea reduction ratios while in the rest, despite comparable urea reductions, NO_x dropped far less and only in a few did levels rise above the predialysis values.[35,77] No differences in ultrafiltrate volume, weight loss, or the reuse of the dialyzer membrane were found to support a mechanical basis for the difference in the plasma NO_x findings. And indeed, systolic BPs fell significantly more in patients with less than 50% change in their plasma NO_x. The duration of the dialysis treatment was shorter and the volume of saline infused was greater in patients with the greater drop in systolic BP, reflecting the clinical severity of the hypotension.[77] Recently, data from a small group of stable subjects who were dialyzed for 3 hours, three times a week, maintaining urea clearance at 65% indicated that the rate of NO synthesis was increased by nearly 50% between the predialysis and the postdialysis phases without the development of hypotension.[97] Thus, even in the absence of hypotension, the process of hemodialysis in stable patients results in increased NO synthesis.

What could be the basis for the increased generation of NO that appears to be the basis for the hypotension developing during hemodialysis?

Collectively, our findings point to the convergence of some of the factors discussed above as the basis for the excessive generation of NO that occurs in patients with symptomatic hypotension. It seems that iNOS induction in the peripheral buffy coat cells occurs in most hemodialyzed patients, but enzyme expression may be masked until inhibitors such as ADMA are removed. The status of iNOS in these cells can be visualized as a model of the status of NOS elsewhere in the patients' bodies. Our study of the activities of buffy coat cells collected serially during hemodialysis showed that expression of NOS activity was detectable within the first hour of dialysis in some where none had been present in the predialysis samples. Similarly, other isoforms of NOS would likely be inhibited until dialysis reduced the inhibitory compounds to a level sufficient to allow NO synthesis to be resumed. In accord with this premise are the findings of higher predialysis systolic BPs (vasoconstriction due to inhibition of NOS) and higher ADMA levels (inhibitor of NOS enzymes) in patients who suffer symptomatic hypotension (less change in plasma NO_x during dialysis) compared to those who do not suffer hypo-

tension (greater drop in plasma NO_x during dialysis) despite comparable urea clearances.

Thus, among the several factors that participate in the increased generation of NO, the level of the NOS inhibitors that accumulate escaping the action of DDAH appears to play an important role. Prior to the next dialysis, high levels of inhibitors diminish the amount of NO produced by endothelial cells causing the overlying smooth muscle cells to contract, maintaining higher predialysis BPs (see Figure 3, upper panel). With reduction of inhibitor levels by dialysis, NO synthesis can be resumed. The effect of shear would also be greater the more constricted the artery, as it would be in patients with the higher predialysis BPs who suffer greater reductions in BP. The strategic location of the endothelial cells adjacent to smooth muscle cells enables NO to rapidly reach its target, to initiate the signal to relax causing vessels to dilate, reducing BPs to hypotensive levels (Figure 3, middle panel). Compensatory vasoconstrictor hormone responses may be offset by a combination of their dilution and removal by dialysis and by tachyphylaxis as patients with hypotension have much higher predialysis BPs and receptors for constrictor hormones may be occupied or internalized.

Clearly, the rbc is not synthesizing NO and hemoglobin appears to play an important role in sequestering NO, possibly attenuating the degree of hypotension that might have otherwise occurred. While DDAH in the circulating rbc and in tissues would affect the concentrations of the methylated arginine inhibitors of NOS that are released during nuclear protein turnover, no defect in DDAH activity was observed in the cohort we studied.

By comparison, when inhibitor levels are low, vascular endothelial cells continue to generate NO at the predialysis rate as modified by stress forces arising from dialysis (Figure 3, lower panel). And, as arterial constriction is far less, shear effects do not rise to that which occurs in patients with higher predialysis BPs.

What about the von Bezold-Jarisch reflex? Up to the moment of the development of symptoms of hypotension, cardiovascular responses appear similar after which hypotension and bradycardia occur[149] as though the cardiac pacemaker were hyperresponsive to cholinergic stimuli.[151] Interestingly, rats made hypertensive with L-NAME exhibit exaggerated von Bezold-Jarisch reflexes.[152] As shown in Figure 4, heart rate responses in a previously reported study are clearly abnormal in patients with $>30\%$ drop in BP during hemodialysis showing bradycardia and insignificant rise compared to patients with $<30\%$ drop in BP where a significant increase can be measured during the same time frame. Interestingly, predialysis pulses were significantly higher in patients with the $>30\%$ drop in BP than those with less of a hypotensive response. And, higher pulses correlated directly with the concentrations of the NOS inhibitor, ADMA.[35]

Given the findings reported here and the state of knowledge of the underlying cause(s) of hemodialysis hypotension, what steps could be taken to reduce the occurrence of symptomatic hypotension during hemodialysis? First, achieving the most thorough dialysis on a consistent basis would lower body levels of inhibitors at the end of each dialysis, reducing basal levels for interim build-up. Second, minimizing catabolic stress which would reduce the rate of nuclear protein turnover would diminish the release of these compounds while maintaining adequate nutrition. Third, the careful use of metal-containing medications, especially iron, might prevent inhibition of DDAH activity, allowing the inhibitors that are released during protein turnover to be kept in check by enzymatic hydrolysis. And finally, the incorporation of means of monitoring these patients during dialysis with high degrees of reliability in detecting changes that are predictive of the likelihood of hypotensive responses to occur.

New approaches are under investigation to evaluate cardiac function as a predictor of

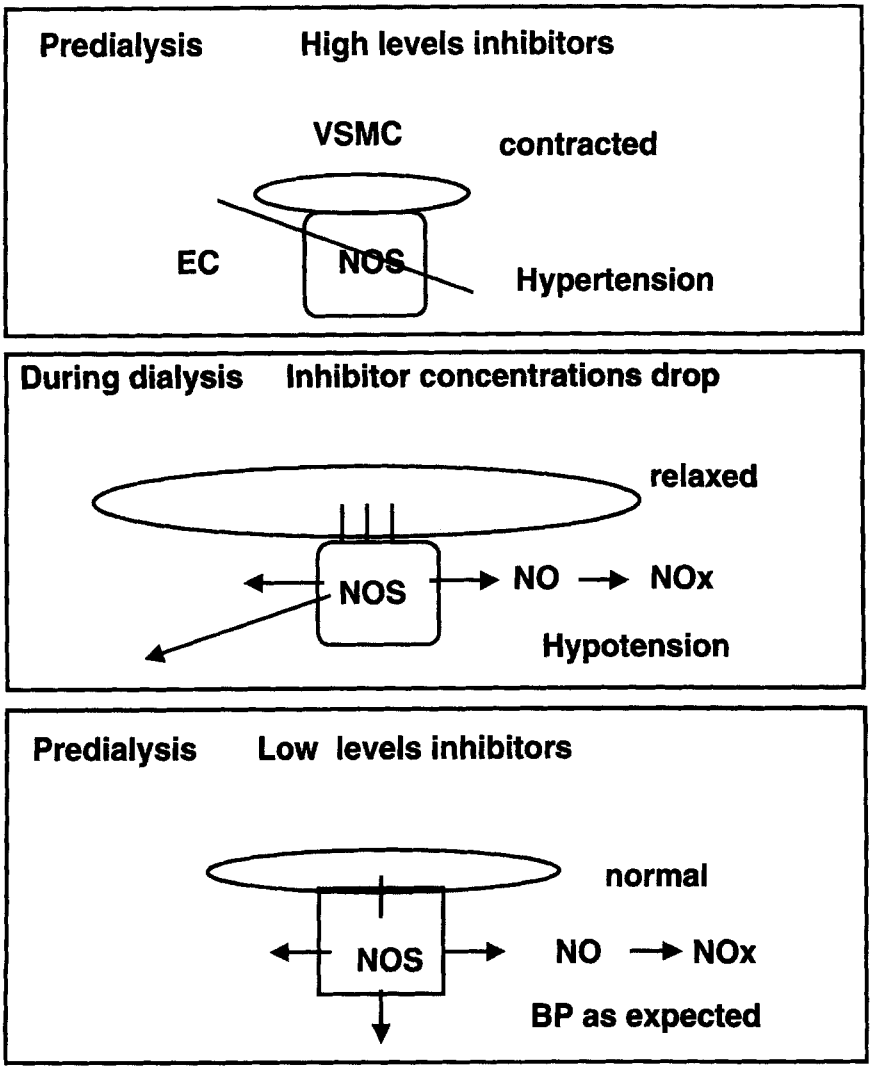

FIGURE 3 Hemodialysis hypotension. Upper panel represents the predialysis status of ESRD patients with high levels of NOS inhibitors, where EC synthesis of NOS is reduced, VSMCs are contracted and hypertension is present. Middle panel represents the resumption of NO synthesis during dialysis when inhibitor levels are reduced, resulting in hypotension. The lower panel represents the predialysis status of ESRD patients with low or normal levels of NOS inhibitors, where EC synthesis of NOS is normal.

hypotension during dialysis as well as to monitor each dialysis procedure by several means to identify patterns and changes that portend the development of severe, sustained hypotension. Among these is the use of ultrasonic pulsed Doppler to measure right and left ventricular inflow and outflow waveforms immediately before dialysis. Indices developed for the right and left heart systems can identify those likely to develop hypotension as having higher left heart system indices before dialysis.[162] Devices that can continuously and noninvasively measure hematocrit and plasma protein concentration during the treatment are being tested.[163] Through these devices, intradialytic changes in blood volume can be fedback to profile or vary the ultrafiltration rate and dialysate sodium concentration. To monitor intradialytic sodium

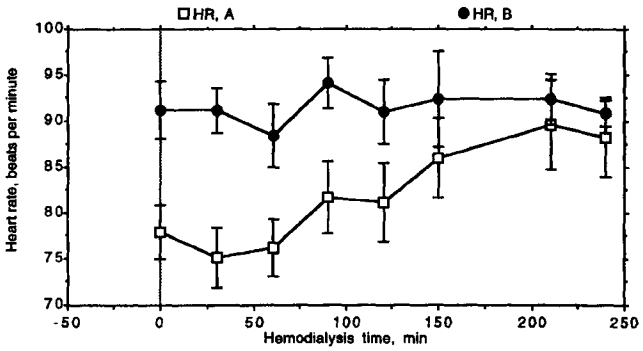

FIGURE 4 Serial pulses during hemodialysis in ESRD patients with < or > 30% drop in systolic BP. Heart rate (mean ± sem) during 12 hemodialyses in Group B where systolic BPs dropped 30% or more represented by the closed circles and in 18 dialyses in Group A where the drop was <30%, represented by the open squares. Analysis of variance, $p = .001$, within 99% confidence limit (Fisher test, 6.179; Scheffe F-test, 28.579).

removal, on-line monitoring has been tested and shown to a reliable and inexpensive method of matching intradialytic sodium removal and the interdialytic sodium load.[164] Together with the use of a plasma to dialysate potassium gradient, these devices appear to reduce the occurrence of symptomatic hypotension.[164,165] The use of hydroxyethylstarch to preserve the blood volume during combined ultrafiltration and hemodialysis is as effective in maintaining systolic BP as the use of albumin.[166,167] The rate of intercompartmental fluid volume changes during hemodialysis must be a major determinant of dialysis-induced hypotension. Plasma refilling calculated by changes in hematocrit, ultrafiltration rates and inferior caval diameter have been reported to be of limited use in the prevention of dialysis-related hypotension.[168] Recently, monitoring volume changes in the peripheral tissues by A-mold ultrasound has been shown to allow a direct and noninvasive means to monitor volume changes during hemodialysis.[169]

Incorporation of such monitoring devices in our treatment regimen, together with extensions of investigations into the status and changes in the generation of the various reactive oxygens that arise during hemodialysis in ESRD patients could eventually lead to the elimination of the occurrence of symptomatic hypotensive episodes.

References

[1] L.W. Henderson (1980) Symptomatic hypotension during dialysis. *Kidney International*, **17**, 571–576.
[2] W.L. Henrich (1986) Hemodynamic instability during hemodialysis. *Kidney International*, **30**, 605–612.
[3] R.L. Converse, Jr., T.N. Jacobsen, C.M.T. Jost, R.D. Toto, P.A. Grayburn, T.M. Obregon, F. Fouad-Tarazi and R.G. Victor (1992) Paradoxical withdrawal of reflex vasoconstriction as a cause of hemodialysis-induced hypotension. *Journal of Clinical Investigation*, **90**, 1657–1665.
[4] J.T. Daugirdas (1991) Dialysis hypotension: a hemodynamic analysis. *Kidney International*, **39**, 233–246.
[5] K.M.L. Leunissen, T.C. Noordzu and J.P. van Hooff (1990) Pathophysiologic aspects of plasma volume preservation. *Contributions in Nephrology*, **78**, 201–211.
[6] S. Malik, R.J. Winney and D.J. Ewing (1986) Chronic renal failure and cardiovascular autonomic function. *Nephron*, **43**, 191–195.
[7] A.S. Nies, D. Robertson and W.J. Stone (1979) Hemodialysis hypotension is not the result of uremic peripheral autonomic neuropathy. *Journal of Laboratory and Clinical Medicine*, **94**, 395–402.
[8] K. Yokokawa, R. Mankus, M.G. Saklayen, M. Kohno, K. Yasunari, M. Minami, H. Kano, T. Horio, T. Takeda and A.K. Mandel (1995) Increased nitric oxide production in patients with hypotension during hemodialysis. *Annals of Internal Medicine*, **123**, 35–37.
[9] B. Straver, M. Roggekamp, P. de Bries and P. ter Wee (1998) Systemic vascular resistance in intradialytic hypotension determined by means of impedance cardiography. *Blood Purification*, **16**, 281–289.
[10] J. Lee (2000) Distinguished Lecture: biomechanics of the microcirculation, an integrative and therapeutic perspective. *Annals of Biomedical Engineering*, **28**, 1–13.
[11] S. Moncada, R.M.J. Palmer and E.A. Higgs (1991) Nitric oxide: physiology, pathophysiology and pharmacology. *Pharmacology Reviews*, **43**, 109–142.
[12] S. Moncada (1993) The L-arginine-nitric oxide pathway. *New England Journal of Medicine*, **329**, 2002–2010.
[13] C.A. Dinarello (1992) Cytokines: agents provocateurs in hemodialysis? *Kidney International*, **41**, 683–694.

[14] A. Schrander-v d Meer, P. ter Wee, G. Kan, A. Donker, W. van Dorp and G. Kennemer (1999) Improved cardiovascular variables during acetate free biofiltration. *Clinical Nephrology*, **51**, 304–309.
[15] M. Noris, M. Todeschini, F. Casiraghi, D. Roccatello, G. Martina, L. Minetti, B. Imberti, F. Gaspari, M. Atti and G. Remuzzi (1998) Effect of acetate, bicarbonate dialysis, and acetate-free biofiltration on nitric oxide synthesis: implications for dialysis hypotension. *American Journal of Kidney Disease*, **32**, 115–124.
[16] M. Nishimura, M. Ushiyama, Y. Maruyama, H. Mabuchi, H. Takahashi and M. Yoshimura (2000) Association of human hepatocyte growth factor with hemodialysis hypotension. *Hypertension Research*, **23**, 581–586.
[17] N. Fernandez, L. Monge, A. Garcia-Villalon, J. Garcia, B. Gomez and G. Dieguez (1994) Cooling effects on nitric oxide production by rabbit ear and femoral arteries during cholinergic stimulation. *British Journal of Pharmacology*, **113**, 550–554.
[18] Q. Maggiore, P. Dattolo, M. Piacenti, M. Morales, G. Pelosi, F. Pizzarelli and T. Cerrai (1995) Thermal balance and dialysis hypotension. *International Journal of Artificial Organs*, **18**, 518–525.
[19] A. Kaufman, A. Morris, V. Lavarias, Y. Wang, J. Leung, M. Glabman, S. Yusuf, A. Levoci, H. Polaschegg and N. Levin (1998) Effects of controlled blood cooling on hemodynamic stability and urea kinetics using high-efficiency hemodialysis. *Journal of the American Society of Nephrology*, **9**, 877–883.
[20] F. Van der Sande, J. Kooman, J. Burema, P. Hameleers, A. Kerkhofs, J. Barendregt and K. Leunissen (1999) Effect of dialysate temperature on energy balance during hemodialysis: quantification of extracorporeal energy transfer. *American Journal of Kidney Disease*, **33**, 1115–1121.
[21] C. Jost, R. Agarwal, T. Khair-el-Din, P. Grayburn, R. Victor and W. Henrich (1993) Effects of cooler temperature dialysate on hemodynamic stability in problem dialysis patients. *Kidney International*, **44**, 606–612.
[22] J.R. Flynn, M. Mitchell, F. Caruso and M. McElligott (1996) MIdodrine treatment for patients with hemodialysis hypotension. *Clinical Nehrology*, **45**, 261–267.
[23] J. Fang and C. Huang (1996) Midodrine hydrochloride in patients on hemodialysis with chronic hypotension. *Renal Failure*, **18**, 253–260.
[24] D. Cruz, R. Mahnensmith, H. Brickel and M. Perazella (1999) Midodrine and cool dialysate are effective therapies for symptomatic intradialytic hypotension. *American Journal of Kidney Disease*, **33**, 920–926.
[25] A. Fine and B. Penner (1996) The protective effect of cool dialysate is dependent on patients' predialysis temperature. *American Journal of Kidney Disease*, **28**, 262–265.
[26] J.M. Lazarus and W.F. Owen (1994) Role of bioincompatibility in dialysis morbidity and mortality. *American Journal of Kidney Disease*, **24**, 1019–1032.
[27] L.W. Henderson, K.M. Koch, C.A. Dinarello and S. Shaldon (1983) Hemodialysis hypotension: The interleukin hypothesis. *Blood Purification*, **1**, 3–8.
[28] F.K. Port, K.M. VanDeKerkhove, S.L. Kunkel and M.J. Kluger (1987) The role of dialysate in the stimulation of interleukin-1 production during clinical hemodialysis. *American Journal of Kidney Disease*, **10**, 118–122.
[29] C.A. Dinarello, K.K. Koch and S. Shaldon (1988) Interleukin-1 and its relevance in patients treated with hemodialysis. *Kidney International*, **33**, S21–S26.
[30] A. Luger, J. Kovarik, H.K. Stummvoll, A. Urbanska and T. Luger (1987) Blood membrane interaction in hemodialysis leads to increased cytokine production. *Kidney International*, **32**, 84–88.
[31] C.A. Dinarello (1992) Interleukin-1 and tumor necrosis factor and their naturally occurring antagonists during hemodialysis. *Kidney International*, **42**, S68–S77.
[32] C. Nathan (1992) Nitric oxide as a secretory product of mammalian cells. *Federation of the American Society for Experimental Biology Journal*, **6**, 3051–3064.
[33] A.K. Nussler and T.R. Billiar (1993) Inflammation, immunoregulation, and inducible nitric oxide synthase. *Journal of Leukocyte Biology*, **54**, 171–178.
[34] D. Beasley and B.M. Brenner (1992) Role of nitric oxide in hemodialysis hypotension. *Kidney International*, **42**, S96–S100.
[35] E.S. Kang, M.T. Tevlin, Y.B. Wang, T.M. Chiang, R. Cardenas, L.K. Myers and S.R. Acchiardo (1999) Hemodialysis hypotension: Interaction of inhibitors, iNOS, and the interdialytic period. *American Journal of the Medical Sciences*, **317**, 1–14.
[36] A. Amore, R. Bonaudo, D. Ghigo, M. Arese, C. Costamagna, P. Cirina, B. Gianoglio, L. Perugini and R. Cappo (1995) Enhanced production of nitric oxide by blood-dialysis membrane interaction. *Journal of the American Society of Nephrology*, **6**, 1278–1283.
[37] M. Noris, A. Benigni, P. Boccardo, S. Aiello, F. Gaspari, M. Todeschini, M. Figliuzzi and G. Remuzzi (1993) Enhanced nitric oxide synthesis in uremia: implications for platelet dysfunction and dialysis hypotension. *Kidney International*, **44**, 445–450.
[38] M. Blum, T. Yachnin, Y. Wollman, T. Chernihovsky, G. Peer, I. Grosskopf, E. Kaplan, D. Silverberg, S. Cabili and A. Iaina (1998) Low nitric oxide production in patients with chronic renal failure. *Nephron*, **79**, 265–268.
[39] S. Lin, P. Chu, F. Yu, L. Diang and Y. Lin (1996) Increased nitric oxide production in hypotensive hemodialysis patients. *American Society for Artificial Internal Organs Journal*, **42**, M895–M899.
[40] F. Port, T. Hulbert-Shearon, R. Wolfe, W. Bloembergen, T. Golper, L. Agodoa and E. Young (1999) Predialysis blood pressure and mortality risk in a national sample of maintenance hemodialysis patients. *American Journal of Kidney Disease*, **33**, 507–517.
[41] O. Ifudu, W. Brezsnyak, C. Reydel, E. McClendon, T. Surgrue, R. DiRienzo, M. Avram and E. Friedman (1995) Pathobiology and functional status of long-term hemodialysis patients. *American Journal of Nephrology*, **15**, 379–385.
[42] N. Powe, B. Jaar, S. Furth, J. Hermann and W. Briggs (1999) Septicemia in dialysis patients: incidence, risk factors, and prognosis. *Kidney International*, **55**, 1081–1090.
[43] B. Jaar, J. Hermann, S. Furth, W. Briggs and N. Powe (2000) Septicemia in diabetic hemodialysis patients: comparison of incidence, risk factors, and mortality with nondiabetic hemodialysis patients. *American Journal of Kidney Disease*, **35**, 282–292.
[44] W. St. Peter, J. Clark and O. Levos (1998) Drug therapy in haemodialysis patients. Special considerations in the elderly. *Drugs in Aging*, **12**, 441–459.
[45] G. Cohen, M. Haag-Weber and W. Horl (1997) Immune dysfunction in uremia. *Kidney International*, **62**, S79–S82.

[46] S. Lew and K. Kaveh (2000) Dialysis access related infections. *American Society for Artificial Internal Organs Journal*, **46**, S6–S12.
[47] J. Ayus and D. Sheikh-Hamad (1998) Silent infection in clotted hemodialysis access graft. *Journal of the American Society of Nephrology*, **9**, 1314–1317.
[48] C. Davis, D. Gretch and R. Carithers (1994) Hepatitis C virus in renal disease. *Current Opinions in Nephrology and Hypertension*, **3**, 164–173.
[49] M. Cendoroglo Neto, S. Manzano, M. Canziani, A. Silva, L. Cirenza, R.d.C. Sesso, H. Ajzen and S. Draibe (1995) Environmental transmission of hepatitis B and hepatitis C viruses within the hemodialysis unit. *Artificial Organs*, **19**, 251–255.
[50] W. Bloembergen and F. Port (1996) Epidemiological perspective on infections in chronic dialysis patients. *Advances in Renal Replacement Therapy*, **3**, 201–207.
[51] G. Sunder-Plassmann, S. Patruta and W. Horl. (1999) Pathobiology of the role of iron in infection. *American Journal of Kidney Disease*, **34**, S25–S29.
[52] A. Saeki, K. Kaito and M. Kobayashi (1996) Impaired neutrophil function in chronic renal failure–dysregulation of surface adhesion molecule expression and phagocytosis. *Nippon Jinzo Gakkai Shi*, **38**, 585–594.
[53] H. Rabb, E. Calderon, P. Bittle and G. Ramirez (1996) Alterations in soluble intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in hemodialysis patients. *American Journal of Kidney Disease*, **27**, 239–243.
[54] H. Rabb, S. Agosti, P. Bittle, M. Fernandez, G. Ramirez and T. Tedder (1995) Alterations in soluble and leukocyte surface L-selectin (CD 62L) in hemodialysis patients. *Journal of the American Society of Nephrology*, **6**, 1445–1450.
[55] S. Heidenreich, M. Schmidt, J. Bachmann and B. Harrach (1996) Apoptosis of monocytes cultured from long-term hemodialysis patients. *Kidney International*, **49**, 792–799.
[56] S. Fishbane (1999) Review of issues relating to iron and infection. *American Journal of Kidney Disease*, **34**, S47–S52.
[57] T. Luscher, Y. Dohi, F. Tanner and C. Boulanger (1991) Endothelium-dependent contol of vascular tone: effects of age, hypertension and lipids. *Basic Research in Cardiology*, **86**, (Suppl. 2), 143–158.
[58] J. Cooke, E. Rossitch, Jr., N. Andon, L. Loscalzo and V. Dzau (1991) Flow activates an endothelial potassium channel to release an endogenous nitrovasodilator. *Journal of Clinical Investigation*, **88**, 1663–1671.
[59] M. Ohno, G. Gibbons, V. Dzau and J. Cooke (1993) Shear stress elevates endothelial cGMP. Role of a potassium channel and G protein coupling. *Circulation*, **88**, 193–197.
[60] W. O'Neill (1995) Flow-mediated NO release from endothelial cells is independent of K^+ channel activation or intracellular Ca^{2+}. *American Journal of Physiology*, **269**, C863–C869.
[61] M. Hecker, A. Mulsch, E. Bassenge and R. Busse (1993) Vasoconstriction and increased flow: to principal mechanisms of shear stress-dependent endothelial autocoid release. *American Journal of Physiology*, **265**, H828–H833.
[62] T. Ziegler, P. Silacci, V. Harrison, D. Hayoz (1998) Nitric oxide synthase expression in endothelial cells exposed to mechanical forces. *Hypertension*, **32**, 351–355.
[63] B. Ranjan, Z. Xiao and S. Diamond (1995) Constitutive NOS expression in cultured endothelial cells is elevated by fluid shear stress. *American Journal of Physiology*, **269**, H550–H555.
[64] D. Lyons, S. Roy, M. Patel, N. Benjamin and C. Swift (1997) Impaired nitric oxide-mediated vasodilatation and total body nitric oxide production in healthy old age. *Clinical Science*, **93**, 519–525.
[65] J. Eschbach, M. Abdulhadi, J. Browne, B. Delano, M. Downing, J. Egrie, R. Evans, E. Friedman, S. Graber, N. Haley, S. Korbet, S. Krantz, A. Lundin, A. Nissenson, D. Ogden, E. Paganini, B. Rader, E. Rutsky, J. Stivelman, W. Stone, P. Teschan, J. van Stone, D. van Wyck, K. Zucherman and J. Adamson (1989) Recombinant human erythropoietin in anemic patients with end-stage renal disease: Results of a phase III multicentere clinical trial. *Annals of Internal Medicine*, **111**, 992–1000.
[66] L. Pereira and C. Mandarim-de-Lacerda (1997) The stereology of the myocardium in rats hypertensive due to the use of an inhibitor of nitric oxide synthesis. *Reviews of Portuguese Cardiology*, **16**, 753–759.
[67] K. Numaguchi, K. Egashira, M. Takemoto, T. Kadokami, H. Shimokawa, K. Sueishi and A. Takeshita (1995) Chronic inhibition of nitric oxide synthesis causes coronary microvascular remodeling in rats. *Hypertension*, **26**, 957–962.
[68] M. Sander, P. Hansen and R. Victor (1995) Sympathetically mediated hypertension caused by chronic inhibition of nitric oxide. *Hypertension*, **26**, 691–695.
[69] J. Coffman (1994) Effects of endothelium-derived nitric oxide on skin and digital blood flow in humans. *American Journal of Physiology*, **267**, H2087–H2090.
[70] P. Kubes and D. Granger (1992) Nitric oxide modulates microvascular permeability. *American Journal of Physiology*, **262**, H611–H615.
[71] I. Kurose, D. Anderson, M. Miyasaka, T. Tamatani, J. Paulson, R. Todd, J. Rusche and D. Granger (1994) Molecular determinants of reperfusion-induced leukocyte adhesion and vascular protein leakage. *Circulation Research*, **74**, 336–343.
[72] A. Moller and P. Grande (1999) Role of prostacyclin and nitric oxide in regulation of basal microvascular hydraulic permeability in cat skeletal muscle. *Journal of Vascular Research*, **36**, 245–252.
[73] R. Scalia, G. Booth and D. Lefer (1999) Vascular endothelial growth factor attenuates leukocyte-endothelium interaction during acute endothelial dysfunction: essential role of endothelium-derived nitric oxide. *Federation of the American Society for Experimental Biology Journal*, **13**, 1039–1046.
[74] A. Salas, M. Sans, A. Soriano, J.C.D. Anderson, J. Pique and J. Panes (2000) Heparin attenuates TNF-alpha induced inflammatory response through a CD11b dependent mechanism. *Gut*, **47**, 88–96.
[75] G. Bugliarello and J. Sevilla (1970) Velocity distribution and other characteristics of steady and pulsatile blood flow in fine glass tubes. *Biorheology*, **7**, 85–107.
[76] G. Cokelet and H. Goldsmith (1991) Decreased hydrodynamic resistance in the two-phase flow of blood through small vertical tubes at low flow rates. *Circulation Research*, **68**, 1–17.
[77] E.S. Kang, S.R. Acchiardo, Y.B. Wang, M.T. Tevlin, T. Hughes and S. Cardoso (1997) Hypotension during

hemodialysis: Role for nitric oxide. *American Journal of the Medical Sciences*, **313**, 138–146.
[78] J.S. Stamler, O.A. Jaraki, J.A. Osborne, D.I. Simon, J.F. Keaney, D.J. Singel, C.R. Valeri and J. Loscalzo (1992) Nitric oxide circulates in mammalian plasma primarily as an S-nitroso adduct of serum albumin. *Proceedings of the National Academy of Science U.S.A*, **89**, 7674–7677.
[79] L. Jia, J. Bonaventura and J.S. Stamler (1996) S-nitrosohaemoglobin: a dynamic activity of blood involved in vascular control. *Nature*, **380**, 221–226.
[80] J.S. Stamler, L. Jia, J.P. Eu, T.J. McMahon, I.T. Demchenko, J. Bonaventura, K. Gernert and C.A. Piantadosi (1997) Blood flow regulation by S-nitrosohemoglobin in the physiological oxygen gradient. *Science*, **276**, 2034–2037.
[81] L.J. Ignarro (1991) Signal transduction mechanisms involving nitric oxide. *Biochemical Pharmacology*, **41**, 485–490.
[82] J. Eschbach (1991) Erythropoietin 1991 – an overview. *American Journal of Kidney Disease*, **18**, 3–9.
[83] A. Raine and S. Roger (1991) Effects of erythropoietin on blood pressure. *American Journal of Kidney Disease*, **18** (4) (Suppl. 1), 76–83.
[84] M. Wolzt, R. MacAllister, D.D.M. Feelisch, S. Moncada, P. Vallance and A. Hobbs (1999) Biochemical characterization of S-nitrosohemoglobin. Mechanisms underlying synthesis, no release, and biological activity. *Journal of Biological Chemistry*, **274**, 28983–28990.
[85] D. Roccatello, G. Mengozzi, B. Alfieri, E. Pignone, E. Menegatti, G. Cavalli, G. Cesano, D. Rossi, M. Formica, T. Inconis, G. Martina, L. Paradisi, L. Sena and G. Piccoli (1997) Early increase in blood nitric oxide, detected by electron paramagnetic resonance as nitrosylhaemoglobin, in haemodialysis. *Nephrology, Dialysis and Transplantation*, **12**, 292–297.
[86] E.S. Kang, D.E. Miles, M.T. Tevlin, T.B. Cates and S.R. Acchiando (2001) Reversible sequestration of nitric oxide by hemoglobin during hemodialysis in end-stage renal disease. *American Journal of the Medical Sciences* (in press).
[87] W. Featherston, Q. Rogers, and R. Freedland (1973) Relative importance of kidney and liver in synthesis of arginine by the rat. *American Journal of Physiology*, **224**, 127–129.
[88] S.N. Dhanakoti, J.T. Brosnan, G.R. Herzberg and M.E. Brosnan (1990) Renal arginine synthesis: studies *in vitro* and *in vivo*. *American Journal of Physiology*, **259**, E437–E442.
[89] H.G. Windmueller and A.E. Spaeth (1981) Source and fate of circulating citrulline. *American Journal of Physiology*, **241**, E473–E480.
[90] O. Levillain, A. Hus-Citharel, F. Morel and L. Bankir (1990) Localization of arginine synthesis along rat nephron. *American Journal of Physiology*, **259**, F916–F923.
[91] N. Bouby, C. Hassler, P. Parvy and L. Bankir (1993) Renal synthesis of arginine in chronic renal failure: *In vivo* and *in vitro* studies in rats with 5/6 nephrectomy. *Kidney International*, **44**, 676–683.
[92] S. Dhanakoti, M. Brosnan, G. Herzberg and J. Brosnan (1992) Cellular and subcellular localization of enzymes of arginine metabolism in rat kidney. *Biochemical Journal*, **282**, 369–375.
[93] W. Chan, M. Wang, J. Kopple and M. Swenseid (1974) Citrulline levels and urea cycle enzymes in uremic rats. *Journal of Nutrition*, **104**, 678–683.
[94] J. Kopple (1978) Abnormal amino acid and protein metabolism in uremia. *Kidney International*, **14**, 340–348.
[95] S. Wassner, J. Bergstrom, S. Brusilow, A. Harper and W. Mitch (1986) Protein metabolism in renal failure: Abnormalities and possible mechanisms. *American Journal of Kidney Disease* **7**, 285–291.
[96] P. De Deyn, B. Marescau, W. Lornoy, I. Becaus and A. Lowenthal (1986) Guanidino compounds in uraemic dialysed patients. *Clinica Chimica Acta*, **157**, 143–150.
[97] T. Lau, W. Owen, Y. Yu, N. Noviski, J. Lyons, D. Zurakowski, R. Tsay, A. Ajami, V. Young and L. Castillo (2000) Arginine, citrulline, and nitric oxide metabolism in end-stage renal disease patients. *Journal of Clinical Investigation*, **105**, 1217–1225.
[98] S. Laidlaw, R. Berg, J. Kopple, H. Naito, W. Walker and M. Walser (1994) Patterns of fasting plasma amino acid levels in chronic renal insufficiency: results from the feasibity phase of the modification of diet in renal disease study. *American Journal of Kidney Disease*, **23**, 504–513.
[99] G. Coles (1972) Body composition in chronic renal failure. *Quarterly Journal of Medicine*, **41**, 25–47.
[100] S. Klahr, A. Levey, G. Beck, A. Caggiula, L. Hunsicker, J. Kusek and G. Striker (1994) The effects of dietary protein restriction and blood-pressure control on the progression of chronic renal disease. *New England Journal of Medicine*, **330**, 877–884.
[101] J. Bergstrom (1995) Why are dialysis patients malnourished? *American Journal of Kidney Disease*, **26**, 229–241.
[102] J. Chiu, B. Wung, J. Shyy, H. Hsieh and D. Wang (1997) Reactive oxygen species are involved in shear stress-induced intercellular adhesion molecule-1 expression in endothelial cells. *Arteriosclerosis, Thrombosis and Vascular Biology*, **17**, 3570–3577.
[103] N. Fukuyama, Y. Takebayashi, M. Hida, H. Ishida, K. Ichimori and H. Nakazawa (1997) Clinical evidence of peroxynitrite formation in chronic renal failure patients with septic shock. *Free Radicals in Biology and Medicine*, **22**, 771–774.
[104] I. Durak, O. Akyol, E. Basesme, O. Canbolat and M. Kavutcu (1994) Reduced erythrocyte defense mechanisms against free radical toxicity in patients with chronic renal failure. *Nephron*, **66**, 76–80.
[105] S. Biasioli, R. Schiavon, E. De Fanti, G. Cavalcanti and D. Giavarina (1996) The role of erythrocytes in the deperoxidative processes in people on hemodialysis. *American Society for Artificial Internal Organs Journal*, **41**, M890–M894.
[106] M. Tepel, M. Echelmeyer, N. Orie and W. Zidek (2000) Increased intracellular reactive oxygen species in patients with end-stage renal failure: effect of hemodialysis. *Kidney International*, **58**, 867–872.
[107] H. Ischiropoulos, L. Zhu, J. Chen, M. Tsai, J. Martin, C. Smith and J. Beckman (1992) Peroxynitrite-mediated tyrosine nitration catalyzed by superoxide dismutase. *Archives of Biochemistry and Biophysics*, **298**, 431–437.
[108] J. Koenig, M. Fischer, E. Bulant, B. Tiran, I. Elmadfa and W. Druml (1997) Antioxidant status in patients on chronic hemodialysis therapy: impact of parenteral selenium supplementation. *Wien Kliniche Wochenschrift*, **17**, 13–19.
[109] E. Peuchant, M. Delmas-Beauvieux, L. Dubourg, M. Thomas, A. Perromat, M. Aparicio, M. Clerc and C. Combe (1997) Antioxidant effects of a supplemented

very low protein diet in chronic renal failure. *Free Radicals in Biology and Medicine*, **22**, 313–320.

[110] S. Biasioli, R. Schiavon, L. Petrosino, L. Cavallini, G. Cavalcanti and E. De Fanti (1998) Dialysis kinetics of homocysteine and reactive oxygen species. *American Society for Artificial Internal Organs Journal*, **44**, M423–M432.

[111] P. Zachee, A. Ferrant, R. Daelemans, L. Coolen, W. Goossens, R. Lins, M. Couttenye, M. De Broe and M. Boogaerts (1993) Oxidative injury to erythrocytes, cell rigidity and splenic hemolysis in hemodialyzed patients before and during erythropoietin treatment. *Nephron*, **65**, 288–293.

[112] N. Vaziri, F. Oveisi and Y. Ding (1998) Role of increased oxygen free radical activity in the pathogenesis of uremic hypertension. *Kidney International*, **53**, 1748–1754.

[113] F. Laurindo, A. Pedro, H. Barbeiro, F. Pileggi, M. Carvalho, O. Augusto and P. da Luz (1994) Vascular free radical release. *Ex vivo* and *in vivo* evidence for a flow-dependent endothelial mechanism. *Circulation Research*, **74**, 700–709.

[114] A. Howard, R. Alexander, R. Nerem, K. Griendling and W. Taylor (1997) Cyclic strain induces an oxidative stress in endothelial cells. *American Journal of Physiology*, **272**, C421–C427.

[115] L. Yeh, P. YJ, J. Hansalia, I. Ahmed, S. Deshpande, P. Goldschmidt-Clermont, K. Irani and B. Alevriadou (1999) Shear-induced tyrosine phosphorylation in endothelial cells requires Rac1-dependent production of ROS. *American Journal of Physiology*, **276**, C838–C847.

[116] B. Gallis, G. Corthals, D. Goodlett, H. Ueba, F. Kim, S. Presnell, D. Figeys, D. Harrison and B. Berks (1999) Identification of flow-dependent endothelial nitric-oxide synthase phosphorylation sites by mass spectrometry and regulation of phosphorylation and nitric oxide production by the phosphatidylinositol 3-kinase inhibitor LY294002. *Journal of Biological Chemistry*, **274**, 30101–30108.

[117] N. Kooy and S. Lewis (1996) Elevation in arterial blood pressure following the development of tachyphylaxis to peroxynitrite. *European Journal of Pharmacology*, **307**, R5–R7.

[118] Y. Kakimoto and S. Akazawa (1970) Isolation and identification of NG,NG- and NG,N′G-dimethylarginine, NG-mono, di- and trimethyllysine, and glucosyl-galactosyl- and galactosyl-d-hydroxylysine from human urine. *Journal of Biological Chemistry*, **245**, 5751–5758.

[119] W. Paik and S. Kim (1971) Protein methylation. *Science*, **1974**, 114–119.

[120] W. Paik and S. Kim (1975) Protein methylation: Chemical, enzymological, and biological significance. *In Advances in enzymology*, Vol. 42 (ed. A. Meister), Wiley, New York, pp. 227–286.

[121] T. Ogawa, M. Kimoto, H. Watanabe and K. Sasaoka (1987) Metabolism of NG,NG- and NG,N′G-dimethylarginine in rats. *Archives of Biochemistry and Biophysics*, **252**, 526–537.

[122] T. Ogawa, M. Kimoto and K. Sasaoka (1987) Occurrence of a new enzyme catalyzing the direct conversion of NG,NG-dimethyl-L-arginine to L-citrulline in rats. *Biochemical Biophysical Research Communications*, **148**, 671–677.

[123] P. Vallance, A. Leone, A. Calver, J. Collier and S. Moncada (1992) Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet*, **339**, 572–575.

[124] R. MacAllister, M. Rambausek, P. Vallance, D. Williams, K. Hoffmann and E. Ritz (1996) Concentrations of dimethyl-L-arginine in the plasmas of patients with end-stage renal failure. *Nephrology, Dialysis and Transplantation*, **11**, 2449–2452.

[125] J. Kielstein, R. Boger, S. Bode-Boger, J. Schaffer, M. Barbey, K. Koch and J. Frolich (1999) Asymmetric dimethylarginine plasma concentrations differ in patients with end-stage renal disease: relationship to treatment method and atherosclerotic disease. *Journal of the American Society of Nephrology*, **10**, 594– 600.

[126] F. Van Der Sande, J. Kooman and K. Leunissen (2000) Strategies for improving hemodynamic stability in cardiac-compromised dialysis patients. *American Journal of Kidney Disease*, **35**, E19.

[127] E. Closs, F. Basha, A. Habermeier and U. Forstermann (1997) Interference of L-arginine analogues with L-arginine transport mediated by the y^+ carrier hCAT-2B. *Nitric Oxide*, **1**, 65–73.

[128] W. Durante, L. Liao and A. Schafer (1995) Differential regulation of L-arginine transport and inducible NOS in cultured vascular smooth muscle cells. *American Journal of Physiology*, **268**, H1158–H1164.

[129] L.W. Henderson, M.E. Miller, R.W. Hamilton and M.E. Norman (1975) Hemodialysis leukopenia and polymorph random mobility – a possible correlation. *Journal of Laboratory and Clinical Medicine*, **85**, 191–197.

[130] P.R. Craddock, J. Fehr, A.P. Dalmasso, K.L. Brigham and H.S. Jacob (1977) Hemodialysis leukopenia: pulmonary vascular leukostasis resulting from complement activation by dialyzer cellophane membranes. *Journal of Clinical Investigation*, **59**, 879–888.

[131] A. Surdacki, M. Nowicki, J. Sandmann, D. Tsikas, R. Boeger, S. Bode-Boeger, O. Kruszelnicka-Kwiatkowska, F. Kokot, J. Dubiel and J. Froelich (1999) Reduced urinary excretion of nitric oxide metabolites and increased plasma levels of asymmetric dimethylarginine in men with essential hypertension. *Journal of Cardiovascular Pharmacology*, **33**, 652–658.

[132] C. Goonasekera, D. Rees, P. Woolard, A. Frend, V. Shah and M. Dillon (1997) Nitric oxide synthase inhibitors and hypertension in children and adolescents. *Journal of Hypertension*, **15**, 901–909.

[133] R. Boger, S. Bode-Boger, W. Thiele, W. Junker, K. Alexander and J. Frolich (1997) Biochemical evidence for impaired nitric oxide synthesis in patients with peripheral arterial occlusive disease. *Circulation*, **95**, 2068–2074.

[134] M. Usui, H. Matsuoka, H. Miyazaki, S. Ueda, S. Okuda and T. Imaizumi (1998) Increased endogenous nitric oxide synthase inhibitor in patients with congestive heart failure. *Life Sciences*, **62**, 2425–2430.

[135] I. Das, N. Khan, B. Puri and S. Hirsch (1996) Elevated endogenous nitric oxide synthase inhibitor in schizophrenic plasma may reflect abnormalities in brain nitric oxide production. *Neuroscience Letters*, **215**, 209–211.

[136] R. Boger, S. Bode-Boger, A. Szuba, P. Tsao, J. Chan, O. Tangphao, T. Blaschke and J. Cooke (1998) Asymmetric dimethylarginine (ADMA): a novel risk factor for endothelial dysfunction: its role in hypercholesterolemia. *Circulation*, **98**, 1842–1847.

[137] T. Ogawa, T.M. Kimoto and K. Sasaoka (1989) Purification and properties of a new enzyme, NG,NG-

dimethylarginine dimethylaminohydrolase, from rat kidney. *Journal of Biological Chemistry*, **264**, 10205–10209.

[138] M. Kimoto, H. Tsuji, T. Ogawa and K. Sasaoka (1993) Detection of NG,NG-dimethylarginine dimethylaminohydrolase in the nitric oxide-generating systems of rats using monoclonal antibody. *Archives of Biochemistry and Biophysics*, **300**, 657–662.

[139] M. Kimoto, S. Miyatake, T. Sasagawa, H. Yamashita, M. Okita, T. Oka, T. Ogawa and H. Tsuji (1998) Purification, cDNA cloning and expression of human NG,NG-dimethylarginine dimethylaminohydrolase. *European Journal of Biochemistry*, **258**, 863–868.

[140] J. Leiper, J. Santa Maria, A. Chubb, R. MacAllister, I. Charles, G. Whitley and P. Vallance (1999) Identification of two human dimethyarginine dimethylaminohydrolases with distinct tissue distributions and homology with microbial arginine deiminases. *Biochemistry Journal*, **343**, 209–214.

[141] R.L. Converse, Jr., T.N. Jacobsen, R.D. Toto, C.M.T. Jost, F. Cosentino, F. Fouad-Tarazi and R.G. Victor (1992) Sympathetic overactivity in patients with chronic renal failure. *New England Journal of Medicine*, **327**, 1912–1918.

[142] G. Ligtenberg, P. Blankestijn, F. Boomsma and H. Koomans (1996) No change in automatic function tests during uncomplicated haemodialysis. *Nephrology, Dialysis and Transplantation*, **11**, 651–656.

[143] B. Straver, P. de Vries, B. ten Voorde, M. Roggekamp, A. Donker and P. ter Wee (1998) Intradialytic hypotension in relation to pre-existent autonomic dysfunction in hemodialysis patients. *International Journal of Artificial Organs*, **21**, 794–801.

[144] G. Pelosi, M. Emdin, C. Carpeggiani, M. Morales, M. Piacenti, P. Dattolo, T. Cerrai, A. Macerata and A. L'abbate (1999) Impaired sympathetic response before intradialytic hypotension: a study based on spectral analysis of heart rate and pressure variability. *Clinical Science*, **96**, 23–31.

[145] A. Cases, N. Esforzado, S. Lario, M. Vera, J. Lopez-Pedret, F. Rivera-Fillat and W. Jimenez (2000) Increased plasma adrenomedullin levels in hemodialysis patients with sustained hypotension. *Kidney International*, **57**, 664–670.

[146] G. Ligtenberg, P. Blankestijn and H. Koomans (1998) Hemodynamic response during lower body negative pressure: role of volume status. *Journal of the American Society of Nephrology*, **9**, 105–113.

[147] M. Ursino and M. Innocenti (1997.) Modeling arterial hypotension during hemodialysis. *Artificial Organs*, **21**, 873–890.

[148] M. Ursino and M. Innocenti (1997) Mathematical investigation of some physiological factors involved in hemodialysis hypotension. *Artificial Organs*, **21**, 891–902.

[149] M. Barnas, W. Boer and H. Koomans (1999) Hemodynamic patterns and spectral analysis of heart rate variability during dialysis hypotension. *Journal of the American Society of Nephrology*, **10**, 2577–2584.

[150] V. Somers and F. Abboud (1996) Neurocardiogenic syncope. *Advances in Internal Medicine*, **41**, 399–435.

[151] M. Araujo, A. Cabral and E. Vasquez (1995) Exaggerated Bezold-Jarisch reflex in the hypertension induced by inhibition of nitric oxide synthesis. *Brazilian Journal of Medicine and Biological Research*, **28**, 1009–1012.

[152] M. Araujo, L. Barker, A. Cabral and E. Vasquez (1998) Inhibition of nitric oxide synthase causes profound enhancement of the Bezold-Jarisch reflex. *American Journal of Hypertension*, **11**, 66–72.

[153] C. Zoccali, G. Tripepi, F. Mallamaci and V. Panuccio (1997) The heart rate response pattern to dialysis hypotension in haemodialysis patients. *Nephrology, Dialysis and Transplantation*, **12**, 519–523.

[154] A. Raine (1996) The susceptible patient. *Nephrology, Dialysis and Transplantation*, **11**, 6–10.

[155] D. Poldermans, A. Man in't Beld, R. Rambaldi, A. Van Den Meiracker, M. Van Den Dorpel, G. Rocchi, E. Boersma, J. Bax, W. Weimar, J. Roelandt and R. Zietse (1999) Cardiac evaluation in hypotension-prone and hypotension-resistant hemodialysis patients. *Kidney International*, **56**, 1905–1911.

[156] M. Hand, W. Haynes and D. Webb (1998) Hemodialysis and L-arginine, but not D-arginine, correct renal failure-associated endothelial dysfunction. *Kidney International*, **53**, 1068–1077.

[157] G. Deliconstantinos, V. Villiotou, J.C. Stavrides, N. Salemes and J. Gogas (1995) Nitric oxide and peroxynitrite production by human erythrocytes: A causative factor of toxic anemia in breast cancer patients. *Anticancer Research*, **15**, 1435–1446.

[158] L.Y. Chen and J.L. Mehta (1998) Evidence for the presence of L-arginine-nitric oxide pathway in human red blood cells: Relevance in the effects of red blood cells on platelet function. *Journal of Cardiovascular Pharmacology*, **32**, 57–61.

[159] B.C. Jubelin and J.L. Gierman (1996) Erythrocytes may synthesize their own nitric oxide. *American Journal of Hypertension*, **9**, 1214–1219.

[160] T. Saheki, Y. Sato, S. Takada and T. Katsunuma (1979) Regulation of urea synthesis in rat liver-inhibition of urea synthesis by L-norvaline. *Journal of Biochemistry*, **86**, 745–750.

[161] E.S. Kang, K. Ford, G. Grokulsky, Y.-B. Wang, T.M. Chiang and S.R. Acchiardo (2000) Normal circulating adult human red blood cells contain inactive NOS proteins. *Journal of Laboratory and Clinical Medicine*, **135**, 444–451.

[162] K. Furukawa, S. Ikeda, T. Naito, Y. Miyahara, T. Iwasaki, T. Matsushita, K. Yakabe, K. Yamaguchi, M. Shikuwa, Y. Muraya and S. Kohno (2000) Cardiac function in dialysis patients evaluated by Doppler echocardiography and its relation to intradialytic hypotension: a new index combining systolic and diastolic function. *Clinical Nephrology*, **53**, 18–24.

[163] J. Leypoldt and R. Lindsay (1999) Hemodynamic monitoring during hemodialysis. *Advances in Renal Replacement Therapy*, **6**, 233–242.

[164] F. Locatelli, C. Manzoni, S. Di Filippo and S. Andrulli (1999) On-line monitoring and convective treatment modalities: short-term advantages. *Nephrology, Dialysis and Transplantation*, **14**, 92–97.

[165] F. Locatelli, S. Andrulli, S. Di Filippo, B. Redaelli, S. Mangano, C. Navino, R. Ariano, M. Tagliaferri, T. Fidelio, M. Corti, S. Civardi and C. Tetta (1998) Effect of on-line conductivity plasma ultrafiltrate kinetic modeling on cardiovascular stability of hemodialysis patients. *Kidney International*, **53**, 1052–1060.

[166] F. Van der Sande, J. Kooman, J. Barendregt, F. Nieman and K. Leunissen (1999) Effect of intravenous saline,

albumin, or hydroxyethylstarch on blood volume during combined ultrafiltration and hemodialysis. *Journal of the American Society of Nephrology*, **10**, 1303–1308.

[167] F. Van der Sande, A. Luik, J. Kooman, V. Verstappen and K. Leunissen (2000) Effect of intravenous fluids on blood pressure course during hemodialysis in hypotensive-prone patients. *Journal of the American Society of Nephrology*, **11**, 550–555.

[168] H.P. Krepel, R.W. Nette, E. Akcahuseyin, W. Weimar and R. Zietse (2000) Variability of relative blood volume during haemodialysis. *Nephrology, Dialysis and Transplantation*, **15**, 673–679.

[169] J. Schumacher, P. Rob, B. Kreft, A. Engelke, M. Heringlake and K.F. Klotz (2000) Measurement of fluid volume shifts during hemodialysis by A-mode ultrasonography. *Blood Purification*, **18**, 103–109.