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**Review**


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# Uremic toxicity: present state of the art

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*ABSTRACT: The uremic syndrome is a complex mixture of organ dysfunctions, which is attributed to the retention of a myriad of compounds that under normal condition are excreted by the healthy kidneys (uremic toxins). In the area of identification and characterization of uremic toxins and in the knowledge of their pathophysiologic importance, major steps forward have been made during recent years. The present article is a review of several of these steps, especially in the area of information about the compounds that could play a role in the development of cardiovascular complications. It is written by those members of the Uremic Toxins Group, which has been created by the European Society for Artificial Organs (ESAO). Each of the 16 authors has written a state of the art in his/her major area of interest. (Int J Artif Organs 2001; 24: 695-725)*

**KEY WORDS:** *Uremia, Uremic toxins, Low molecular weight proteins, AGE, PTH, AOPP, Protein bound uremic solutes, P-cresol, CMPF, Removal strategies*

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## 1) INTRODUCTION (RV)

In 1998, the European Society for Artificial Organs (ESAO) decided to initiate Working Groups in some of the areas of progress in the field of artificial organs, such as artificial liver, artificial heart, bioartificial organs, experimental models and adsorption systems.

Starting in October 1999, the launch of a Uremic Toxins

Working Group was prepared by three members (UB, BS and RV). In September 2000, on the occasion of the 27<sup>th</sup> ESAO Meeting in Lausanne, the group, composed of several European biochemists and clinicians involved in uremic toxicity research, had its first meeting. Twelve members in research at the university level and five of the leading industries in this area (Baxter Healthcare, Bellco, Fresenius Medical Care, Gambro, Membrana) were

involved. Each research group and industry is represented by one of the authors of the present paper, carefully selected in function of their previous accomplishments and/or by the future interest of their topic.

One of the first projects this group agreed upon was to write a paper about the main subject of interest of each member. The present publication is the result of this common effort. Future projects include the publication of a list of uremic toxins with their threshold concentrations for *in vitro* research, the development of a complete issue devoted to comprehensive reviews on all uremic solutes and common research projects.

The present review paper is built around three main topics: 1) specific toxins, 2) specific biochemical effects, mainly regarding cardio-vascular problems, and 3) therapeutic implications. The title of each subsection is followed, between brackets, by the initials of the author(s).

## 2) THE GENERAL PICTURE OF UREMIC TOXICITY (MLW)

There are many proposed uremic toxins. According to classical definitions of toxicology, a uremic toxin should meet the following criteria:

- It must be a chemical or biological agent capable of producing a response
- It must interact with biological systems, and produce a biological response
- The response should be considered deleterious to the biological system

### a) Identification of toxins

The identification of uremic toxins should follow a multi-tiered approach. As a first step, it is necessary to identify compounds increased in end stage renal failure patients, due to failure of the kidney to eliminate these substances, uremia *per se*, or secondary factors (such as pharmaceuticals, extracorporeal therapies). Identification can be a difficult challenge given the vast heterogeneity of various molecules (1-12).

*In vitro* or simple model systems can answer several questions such as: Does an increased concentration of a toxin produce a biological effect? Is the compound reactive? Can it modify proteins, act on receptors, activate cell signaling pathways? Are there other

molecules that can inhibit, neutralize or degrade this toxin? Are these also increased (or decreased) with respect to the toxin?

It is also important to distinguish between uremic markers and toxins. A marker is an indication that "something has happened". It does not imply that the substance is necessarily toxic. Urea can be considered a "marker" for a poor or failing kidney, but has yet to be conclusively demonstrated as a uremic toxin. Markers can be very useful in evaluating certain pathophysiological responses, but care must be taken not to confuse them with toxins; in some cases there is an overlapping continuum in which there is not a clear distinction.

This leads to a second problem in the identification of uremic toxins. Many molecules are highly reactive, or react in such local micro-environments, that they are not measurable in plasma or tissue samples. In this case, we often need to use markers to give us a clue that a reaction has taken place. The problem with markers is that many are rather non-specific and can lead to incorrect interpretations. This problem might become particularly apparent in the use of standard assay methods or commercialized kits, which have not been thoroughly tested in samples from uremic patients.

### b) Interaction of uremic toxins with biological systems

The second step in the process of identifying uremic toxins is more difficult and is concerned with the relation of the toxin to the (patho)-physiological milieu. A toxin can theoretically "trigger" a number of cellular responses but we do not always know whether these are beneficial or detrimental.

For example, apoptosis is beneficial when it occurs during cell housekeeping to remove old or damaged cells, but it could be considered detrimental when inappropriately stimulated to cause premature cell death, or when it is inactivated so that it allows unregulated cell proliferation. Often the answer to these questions depends on the local microenvironment and whether the response is physiologic, an adaptation to chemical or physical stress or an inappropriate hyper- or hypo-response.

Finally we must look at the overall deleterious consequences to the patient, which can be difficult due to complex interactions. A typical example is the interference of various inflammatory mediators with the abnormal immune and inflammatory responses produced as a result of renal failure (13-15).

### 3) SPECIFIC UREMIC TOXINS

In this section, a number of specific toxins will be discussed, together with their origins, characteristics, patho-physiologic aspects and modes of removal. Many of these solutes have been selected in function of their role in cardio-vascular morbidity/mortality.

#### *a) Low molecular weight proteins in general (WC)*

With the identification of  $\beta_2$ -microglobulin ( $\beta_2$ M) as the main protein component of dialysis-related amyloid (DRA) (16), low-molecular weight proteins (LMWPs) became a distinct class of uremic toxins. Since this discovery, a number of other putative uremic toxins from this same category have been identified (17-21). LMWP handling in conditions of renal health and disease, with particular attention to LMWPs of potential clinical significance in uremic patients, is discussed briefly below.

Although not precisely defined, LMWPs as a class have a molecular weight spectrum ranging from approximately 1,000 to 50,000 Daltons. Although the initial step in solute removal by the kidney is glomerular filtration, the net elimination of uremic toxins is primarily determined by processes occurring in distal portions of the kidney. The sequential renal metabolic processes are glomerular filtration and luminal reabsorption at the proximal tubule, with the former as the rate-limiting step. Hydrolysis of the reabsorbed protein to its constituent amino acids happens within the proximal tubular cell, after which anti-luminal reabsorption by the peritubular capillaries occurs.

A prototypical LMWP for which the metabolism in patients with normal renal function and varying degrees of renal insufficiency has been characterized is Complement Factor D, a 23.5 kDa up-regulator of the alternative complement pathway (22). A significant direct correlation was observed between serum Factor D concentration and serum creatinine while the relationship between serum Factor D concentration and creatinine clearance was very similar to that between serum creatinine and creatinine clearance. In subjects with intact glomerular and proximal tubule function, despite a glomerular sieving coefficient of 0.36, less than 0.2% of filtered Factor D was measured in the final urine. In this study, serum Factor D concentrations in patients with end-stage renal disease (ESRD) were 10-20 fold higher than in those with normal kidney function.

In some of the following sections (3b, 3c and 3d), more specific attention will be paid to a number of individual

peptidic molecules, such as  $\beta_2$ -microglobulin, leptin and several low molecular weight molecules with modulating properties on leukocyte functions.

#### *b) $\beta_2$ -microglobulin (AA)*

In 1980, Assenat et al identified amyloid deposits in the carpal tunnel of dialysis patients (23). In 1985 Gejyo et al demonstrated that the main protein component of dialysis related amyloidosis is  $\beta_2$ -microglobulin (16).  $\beta_2$ -microglobulin amyloidosis mainly involves bone, tendons and joints but can also be systemic (24-29). Bone lesions are frequently observed in the carpal tunnel as well as in long bones and spine (30-34). Carpal and scapulo-humeral joints are the most frequently involved joints (35). Coxo-femoral (36), popliteal (37) and many other localisations have also been reported (38-40).  $\beta_2$ -microglobulin amyloidosis has a very high prevalence, close to 100% after 15 years of dialysis (41), although according to more recent studies the prevalence might be decreasing (42).

The pathophysiology of  $\beta_2$ -microglobulin amyloidosis is unknown. The main factors believed to participate in its genesis are proteins and cells. The proteins involved in  $\beta_2$ -microglobulin amyloidosis include  $\beta_2$ -microglobulin,  $\alpha_2$ -macroglobulin, globin and light chains of immunoglobulins, amyloid P component and others (38). Macrophages are the main cell type found in amyloid deposits (43).

Patients with  $\beta_2$ -microglobulin amyloidosis, nearly exclusively dialysis patients, invariably have high serum levels of  $\beta_2$ -microglobulin. However, many studies have demonstrated that there is no relationship between serum levels of  $\beta_2$ -microglobulin and the incidence of  $\beta_2$ -microglobulin amyloidosis (44-46). Therefore, although it might be "politically correct" to aim to decrease  $\beta_2$ -microglobulin serum levels (47), such a strategy has not been proven to prevent or reverse  $\beta_2$ -microglobulin amyloidosis. Even after a successful kidney trans-plantation with a near normalisation of serum levels of  $\beta_2$ -microglobulin, there is no regression of the amyloid deposits (48).

Based on the study by van Ypersele de Strihou et al (49) some authors have advised the use of synthetic bio-compatible membranes to prevent or delay  $\beta_2$ -microglobulin amyloidosis (50-52). However, other reports have not confirmed these results (42, 53, 54).

Several modifications of  $\beta_2$ -microglobulin have been proposed as participating in the formation of amyloid fibrils: proteolysis of the N-terminus of  $\beta_2$ -microglobulin (55, 56), deamidation of the Asn17 (57, 58) and Advanced

Glycation End Product (AGE) modification of  $\beta_2$ -microglobulin (59). However, proteolysis is usually not observed in amyloid deposits (31,60-63), the more acidic isoforms of  $\beta_2$ -microglobulin are not necessarily deamidated in the 17<sup>th</sup> (62, 64), and there is evidence that AGEs are not a primary event in the formation of  $\beta_2$ -microglobulin amyloid deposits (65).

The other protein components of  $\beta_2$ -microglobulin amyloidosis may also play a role in the pathogenesis of this disease. Particularly important would be  $\alpha_2$ -macroglobulin (66), glucosaminoglycans (67-70) and amyloid P component (71-74).

Since macrophages are the main cellular type present in amyloid deposits, it seems important to establish whether they participate in the formation of amyloid fibrils or whether they are rather a part of the response mechanisms to the presence of amyloidosis. Our studies with ultrastructural immunogold labelling and more recently those of Garbar et al based on systematic histology of dialysis patients, suggest that macrophage infiltrates are a secondary phenomenon (75, 76).

In conclusion, since the pathogenesis of dialysis related amyloidosis has not been completely elucidated, many candidate substances deserve study to improve our understanding of the disease and to find preventive measures.

### *c) Leptin (PS)*

The discovery of the *ob* gene product leptin (16 kD) has increased our understanding of the physiological system that regulates eating behavior. Recent studies have demonstrated that most, but not all, ESRD patients have inappropriately high leptin levels (77, 78). It has been speculated that leptin may be a uremic toxin that mediates anorexia and wasting (79). Serum free leptin levels are elevated whereas serum bound leptin levels remain stable in ESRD patients (80). Besides regulating appetite, leptin may also play a role in insulin metabolism, sodium handling, hematopoiesis and bone formation in ESRD patients (81, 82). As the kidneys clear other polypeptide hormones, it seems reasonable to surmise that leptin also accumulates in the case of renal failure due to reduced renal clearance. Indeed, the kidney is the principal site of elimination of circulating leptin in healthy subjects (17). Moreover, an inverse correlation between leptin and GFR has been demonstrated in patients with various degrees of renal failure (83) and, in rats, bilateral nephrectomy

reduces plasma leptin clearance by 80% (84). Recent *in vivo* studies in rats show that uptake and degradation of leptin by renal tissue is the main mechanism of elimination (85). The important role of the kidney in leptin metabolism is further underscored by the fact that renal transplantation normalizes leptin levels (86).

However, not all ESRD-patients have elevated leptin levels (87, 88) and some patients even have low leptin levels suggesting that other tissues such as splanchnic organs contribute to leptin removal (89). Moreover, as ESRD patients have lower leptin mRNA than controls, this could suggest that decreased plasma leptin clearance is a part of the efferent feedback loop that down-regulates the expression of the *ob* gene in hyperleptinemic ESRD patients (83). However, also other factors associated with ESRD, such as hyperinsulinemia and inflammation, may affect leptin levels (81, 87). In animal models, elevated serum leptin levels may be responsible for the anorexia observed during inflammatory conditions (90, 91). Also in humans, the administration of cytokines, such as IL-1 $\beta$  and TNF- $\alpha$ , has been shown to increase serum leptin levels (92, 93). However, a recent study by Don et al in ESRD-patients suggests that leptin levels may be suppressed during inflammation and may actually act as a negative acute phase reactant. Hence, it is obvious that more human studies are needed (94). Finally, it should be pointed out that female gender and obesity are important factors that also affect serum leptin levels in ESRD patients (81).

Although there is as yet no direct evidence that increased levels of leptin cause anorexia, some indirect evidence suggests that leptin may mediate anorexia in ESRD patients (79, 95, 96). Moreover, it has been shown that high levels of serum leptin relative to fat mass might be associated with weight loss in HD patients (97) and increasing serum leptin levels are associated with a loss of lean body mass in CAPD patients (98). However, as others find no association between the leptin concentration and recent change in weight or nutritional status in ESRD (78,99), the question whether or not elevated serum leptin levels cause anorexia is open.

In conclusion, although hyperleptinemia is a common phenomenon in ESRD patients, it is not a ubiquitous finding. Moreover, although some indirect evidence suggests that elevated leptin levels might cause anorexia, not all studies have found such a relationship. Accordingly, on the basis of present knowledge, leptin can only be regarded as a suspected uremic toxin (Tab. I).

d) Immune modulating proteins (GC)

The increased incidence of infections among patients with chronic renal failure is primarily the consequence of disturbed functions of polymorphonuclear leukocytes (PMNL), which are cells that take care of the first-line unspecific immune defense. A perfect balance between stimulation and inhibition is necessary to maintain an effective immune response without harmful side effects on PMNL action. Furthermore, the clearance of PMNL via apoptosis from the site of infection is crucial for the coordinated resolution of inflammation. The apoptotic cell death of PMNL is regulated by pro- and anti-apoptotic factors.

During the last few years, a number of peptides that accumulate in sera from uremic patients have been purified from hemodialysis ultrafiltrate and CAPD effluents and their effect on PMNL has been studied using *in vitro* assays. A granulocyte inhibitory protein (GIP I) with homology to free immunoglobulin light chains (IgLCs) interferes with several crucial PMNL functions including chemotaxis, glucose uptake, oxidative metabolism, and phagocytosis (18). GIP II, a granulocyte inhibitory protein with homology to  $\beta_2$ -microglobulin, inhibits deoxyglucose uptake and PMNL oxidative metabolism stimulated by phorbol-myristate-acetate (100). Angiogenin isolated from uremic patients has been identified as a degranulation inhibitory protein (DIP I) (101). Whereas DIP I inhibits the release of collagenase, gelatinase, and lactoferrin in nanomolar concentrations, it has no effect on other PMNL functions such as chemotaxis, phagocytosis, and oxidative metabolism. Another degranulation inhibitory protein (DIP II) has been isolated from human plasma ultrafiltrate and has been identified as complement factor D (102). Recently, a chemotaxis inhibiting peptide with homology to

ubiquitin has been isolated from the peritoneal effluent of CAPD patients and from hemodialysis ultrafiltrate (19).

Immunoglobulin light chains (IgLCs) are produced by B cells slightly in excess to Ig heavy chains (103). A small amount of free LCs is present in normal serum. Compared to healthy controls, the plasma levels of free immunoglobulin light chains are elevated in chronic renal failure patients (104) and are even higher in patients undergoing hemodialysis treatment (105). Free IgLCs which are isolated from hemodialysis and continuous ambulatory peritoneal dialysis patients inhibit PMNL chemotaxis and the stimulation of glucose uptake (106). On the other hand, IgLCs stimulate basal levels of glucose uptake and the oxidative metabolism of PMNL (107). Furthermore, the presence of free IgLCs increases the percentage of viable PMNL by inhibiting spontaneous apoptotic cell death (108). Therefore, in uremia, IgLC could not only contribute to the diminished immune function upon stimulation possibly related to infectious susceptibility but also to the state of baseline pre-activation which could play a role in atherogenesis (109).

e) Advanced glycation end products (AGE) (TH,GC)

The Maillard reaction between reducing carbohydrates and amino compounds was well studied for food systems during the last century, and has attracted new interest due to detection of its reaction products in human tissues over the last twenty years (110). The amount of several amino acid derivatives of such non-enzymatic glycation of individual amino acids, peptides or proteins (the so-called advanced glycation end products (AGEs)), was found to be increased *in vivo* during aging and diabetes (111, 112). The level of glucose-modified proteins is elevated in uremic patients and is even higher than in diabetic patients

**TABLE I - DOES SERUM LEPTIN CONFORM TO THE CRITERIA FOR A UREMIC TOXIN?**

		Apply to leptin
1	The compound should be chemically identified and accurate quantitative analysis in biologic fluids should be possible.	Yes
2	The plasma level of the compound should be higher in uremic than non-uremic patients.	No, not all uremic patients have elevated serum-leptin
3	High concentrations should be related to specific uremic symptoms that decrease or disappear when the concentration is reduced.	Not yet proven
4	When studying toxicity of specific compounds in human subjects, experimental animals and/or in appropriate <i>in vitro</i> systems, the concentrations used should be comparable to those found in body fluid and/or tissue of uremic patients.	Yes

without renal disease (113). As a significant accumulation of AGEs was observed in both diabetic and non-diabetic patients with renal disease, various complications of the uremic syndrome, such as beta-2-microglobulin-derived amyloidosis, endothelial dysfunction, accelerated atherogenesis and dyslipidemia, have been directly related to the decreased renal elimination of these amino acid derivatives (114-117). For CAPD patients, deposition of AGEs in the peritoneal basement membrane correlates with impairment of peritoneal permeability (118). On a cellular level, AGE-induced effects include stimulation of cytokine synthesis by monocytes, interaction with a specific receptor for AGEs (RAGE), increase of oxidative stress, quenching of nitric oxide and inhibition of nitric oxide synthases (119-124).

Serum proteins from healthy donors have been modified by glucose *in vitro* (125). The extent of early glycation was monitored by boronate chromatography and fructosamine assay; the extent of late glycation was assessed by fluorescence spectroscopy and Western blotting using a specific antibody for imidazolone. By adding amino-guanidine, a glycation inhibitor, to part of the *in vitro* incubations, only protein samples with early glycation were obtained. Late glycation products have been shown to increase PMNL chemotaxis compared to unmodified proteins (125). In contrast, the presence of early glycation of proteins induced an increase of glucose uptake and an accelerated apoptotic cell death of PMNL (125). This suggests that glucose-modified proteins contribute to the enhancement of PMNL functional activities at an early phase leading to functional impairment in a later phase.

Today, AGE proteins and peptides are generally accepted as an important class of "uremic toxins" (3, 14, 126). However, the main questions are still unresolved. As immunological determination of AGEs is mainly achieved using antibodies which have been raised to "AGE-proteins" with poorly characterized chemical composition (113, 127), the most important enigma is the chemical basis of "AGE" and the quantitative contribution of individual compounds. More specific but quite laborious chromatographic methods (HPLC, GC/MS) have only been used in a limited number of reports for AGE analysis. None of the AGEs quantified as of today (Fig. 1), namely fructoselysine, carboxymethyllysine, pyrroline, pentosidine and two imidazolium crosslinks (128-131) (glyoxal-lysine dimer (GOLD); methylglyoxal-lysine-dimer (MOLD)), have yet been directly linked to specific biological effects on a

molecular or cellular basis. AGEs have been localized on atheromatous arteries from non-diabetic (132,133) and diabetic patients (134). However, there is no observational study examining the relationship between AGEs and vascular disease in the general population. Furthermore, the amino acid derivatives that are known today represent only a small fraction of all the AGE compounds generated by the interaction of amino acids with carbonyl compounds (135) (Fig. 2). Do the known compounds really act as uremic toxins, or might there be other, up to now overlooked derivatives with more pronounced toxicity? Which of the circulating AGEs do interact with RAGE or exhibit defined toxic effects? Finally, can "AGE-toxicity" be prevented by optimized therapeutic strategies, e.g. removal via dialysis or pharmacological means?

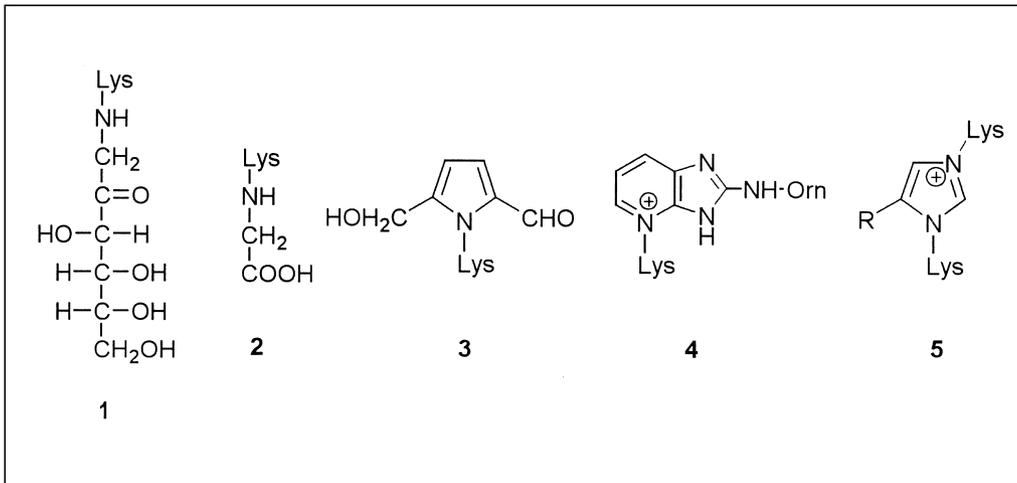
It is well known in the field of food science, that during industrial processes or home-cooking as well as during long-term storage of foods, side-chain modifications by carbohydrates can significantly influence the functional and nutritional properties of food proteins (151, 152). With respect to the extent of lysine and arginine modification in heated or stored foods, questions arise concerning the daily nutritional intake of AGEs and their possible (patho)physiological role (127, 153). The amount of specific amino acid derivatives ingested with meals that originate from certain heated foods can be more than ten times higher than the total amount of AGEs in the uremic body. Preliminary studies indicate that dietary AGEs might contribute significantly to the total AGE-load of the body (153). Physiological consequences resulting from the ingestion of certain foods must therefore be considered within the context of the "AGE discussion".

To answer the above questions concerning the role of AGEs as uremic toxins, an interdisciplinary approach is necessary, joining knowledge, competence and the methods of medicine, biology and food chemistry. This should finally lead to a more comprehensive understanding of a topic, from which to date only the tip of the iceberg is known.

#### *f) Parathyroid hormone (MR)*

Uremic patients frequently develop secondary hyperparathyroidism. It is generally accepted that this results from a persistent demand on the parathyroid cells directly derived from the inability of the uremic organism to maintain an appropriate control of serum calcium (154).

High serum phosphate, vitamin D deficiency and



**Fig. 1** - AGEs quantified in biological specimens: **1**, fructoselysine (136); **2**, N-ε-carboxymethyllysine (131); **3**, pyrraline (137); **4**, pentosidine (138); **5**, GOLD (R = H, glyoxal-lysine-dimer), MOLD (R = CH<sub>3</sub>, methylglyoxal-lysine-dimer) (129).

hypocalcemia are the key factors involved in the pathogenesis of secondary hyperparathyroidism (155). In addition, as a consequence of uremia and/or secondary hyperparathyroidism, a number of cell receptors including PTH/PTHrP, vitamin D receptor (VDR) and calcium receptor (Ca R) are either downregulated or show an abnormal function (155). Thus, abnormalities in receptors are cause and consequence of hyperparathyroidism and uremic toxins other than parathyroid hormone (PTH) are responsible for abnormal receptor function (156,157).

In uremic hyperparathyroidism, the high levels of PTH are usually associated to low serum levels of calcitriol, hypocalcemia and hyperphosphatemia; therefore, it is difficult to separate the undesirable effects of high PTH from those of low calcitriol, low serum calcium and high serum phosphate. Nevertheless it may be reasonable to distinguish three main clinical problems of uremic patients which are related to secondary hyperparathyroidism (Tab. II): osteodystrophy (158), cardiovascular disease including vascular calcifications (159,160) and to some extent alterations in the immune system (161). These are relevant problems which directly affect the life expectancy of dialysis patients.

Hyperphosphatemia is not only a cause of hyperparathyroidism (162) but is also the result of the action of PTH on the bone; furthermore the administration of calcitriol in an attempt to control PTH produces hyperphosphatemia (163).

Hyperphosphatemia and an increased calcium-phosphate product are major patho-physiologic factors responsible for the development of vascular calcifications and increased mortality of ESRD-patients (Tab. III) (164, 165).

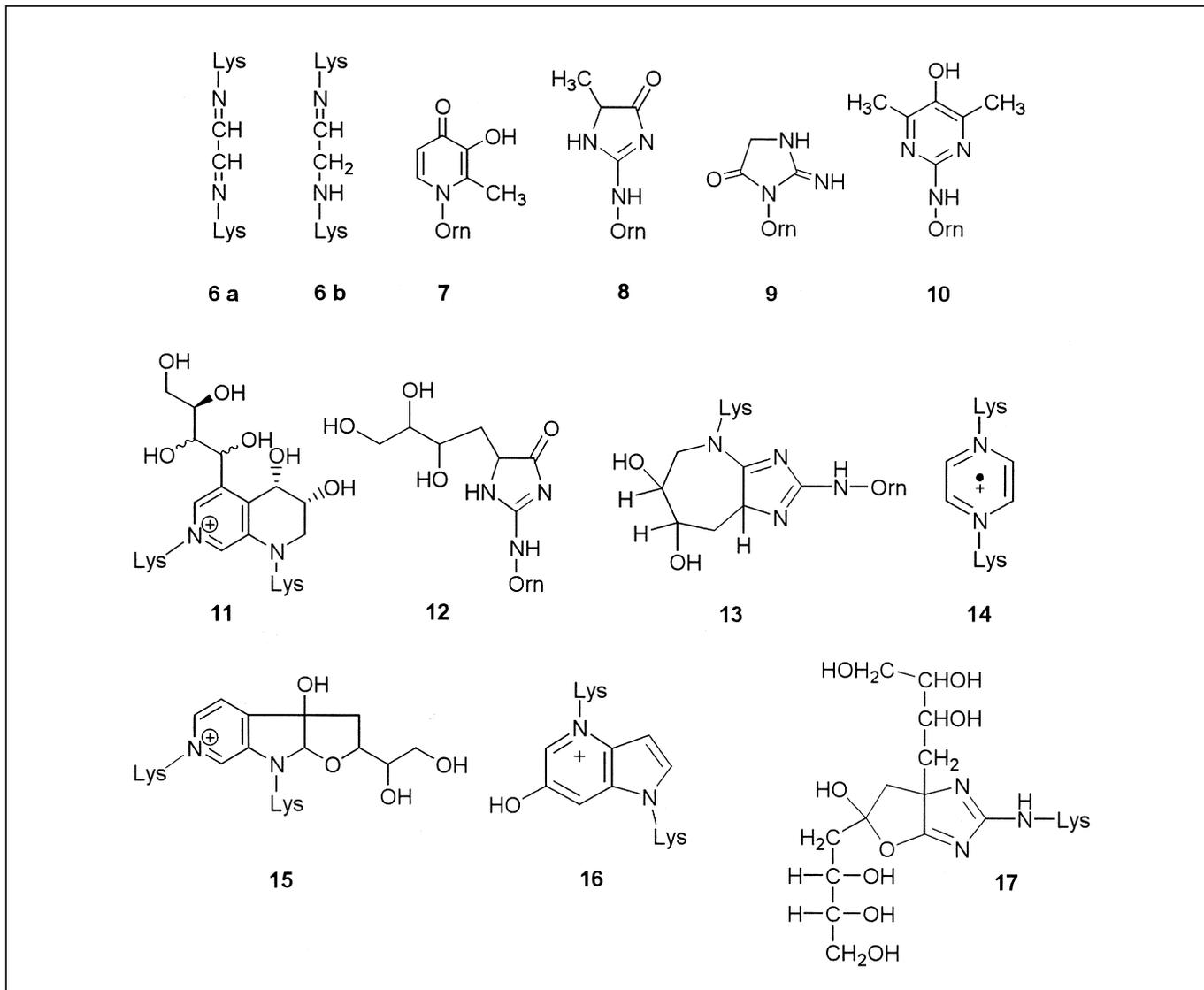
Calcium may not be considered as a uremic toxin since hypercalcemia in uremia is a direct consequence of tertiary hyperparathyroidism, calcium carbonate administration (as treatment of hyperphosphatemia), adynamic bone (due to age, diabetes mellitus, calcitriol administration and relatively low PTH levels) and calcitriol administration (as treatment of uremic hyperparathyroidism). Nevertheless hypercalcemia together with hyperphosphatemia promotes calcification.

Calcitriol should not be considered as a uremic toxin since it increases only after exogenous administration; nevertheless, it may cause hyperphosphatemia and hypercalcemia.

Traditionally, serum phosphate levels below 5.5 mg/dl have been considered acceptable, although this threshold has recently been challenged, since slightly increased serum phosphate levels may counteract an appropriate control of PTH and more importantly, they may increase mortality as well. Thus serum phosphate in a hemodialysis patient should be maintained within the normal limits.

Intact PTH levels below the upper normal limit (60 pg/ml) have been associated to adynamic bone suggesting that in order to have a normal bone turnover the uremic patient requires intact PTH levels 2-3 times above the upper normal limit (158).

The strategies to prevent secondary hyperparathyroidism include vitamin D analogues and reduction of intestinal phosphate absorption using calcium salts. For the treatment of secondary hyperparathyroidism, the reduction of serum phosphate levels using phosphate binders and dietary counseling is mandatory (166). A calcium free phosphate binder (Renagel®) is now commercialised with promising results and other calcium free phosphate binders



**Fig. 2 - Further candidates for AGE-structures (selected examples): 6, glyoxal-lysine-imines (139); 7, maltosine (140); 8, ornithino-methylimidazolinone (141); 9, glarg (142); 10, argpyrimidine (143); 11, crossline (144); 12, "imidazolone" (145); 13, glucosepan (146); 14, crosspy (147); 15, lysylpyrropropyridin (148); 16, vesperlysine (149); 17, dehydrofuroimidazole (150).**

**TABLE II - DOES SERUM PTH CONFORM TO THE CRITERIA FOR A UREMIC TOXIN?**

		Apply to PTH
1	The compound should be chemically identified and accurate quantitative analysis in biologic fluids should be possible.	Yes
2	The plasma level of the compound should be higher in uremic than non-uremic patients.	Yes
3	High concentrations should be related to specific uremic symptoms that decrease or disappear when the concentration is reduced.	Yes for osteodystrophy, not always for calcifications or cardiovascular disease
4	When studying toxicity of specific compounds in human subjects, experimental animals and/or in appropriate <i>in vitro</i> systems, the concentrations used should be comparable to those found in the body fluids and/or tissue of uremic patients.	Yes

might be developed in the future (167); new vitamin D analogues which seem to have less calcemic and phosphatemic effect are under development (168). Finally, calcimimetics, which decrease PTH secretion by acting on the calcium receptor are evaluated in clinical trials. In the near future, calcimimetics may become a new modality of treatment for secondary hyperparathyroidism (169). Nevertheless, parathyroidectomy is a valuable alternative when medical treatment fails.

The cellular and the tissular effects and the clinical consequences of high PTH and hyperphosphatemia are widely recognized by the nephrologist. The future task for the clinician-investigator is: a) to define mechanisms and factors which contribute to the tissue damage induced by high PTH and phosphate; b) to identify new tools and improve old strategies for the control of PTH and hyperphosphatemia; c) to propose a "realistic approach" for the prevention of hyperphosphatemia and hyperparathyroidism.

*g) Reactive carbonyl compounds (AJ)*

In uremia, an accumulation occurs of small-molecular reactive carbonyl compounds, which are derived from the metabolism of carbohydrates and polyunsaturated fatty acids (170, 171). These include glyoxal, methylglyoxal and 3-deoxyglucosone (Tab. IV) (172). It is unclear at present whether the increased concentrations of carbonyl compounds in uremic serum are a consequence of increased formation, reduced clearance, or both. In hemodialysis patients, increased carbonyl formation might be partially due to "oxidative stress" caused by an augmented production of oxidants and a decreased level of antioxidants (171). This might be worsened by the hemodialysis procedure itself since

blood-membrane interactions may lead to complement and leukocyte activation as a consequence of the increased formation and release of reactive oxygen species. On the other hand, carbonyl compounds derived from the metabolism of carbohydrates may be of particular importance in patients on long-term peritoneal dialysis since commercial peritoneal dialysis solutions contain considerable amounts of aldehydes and dicarbonyls as a consequence of glucose degradation during heat sterilisation (173). Recently, considerable amounts of total (non-glucose) carbonyls were detected in both plasma and peritoneal effluent of CAPD patients (174).

The biological consequences of increased reactive carbonyl compounds might either be related to their direct toxicity or indirectly, i.e. via the increased formation of advanced glycation (AGE) or lipoxidation (ALE) end products (171). The hypothesis of direct toxicity is supported by data showing that heat-sterilized peritoneal dialysis fluids interfere with cell function (L929, leukocytes, peritoneal mesothelial cells) to a much larger extent than filter-sterilized fluids (175, 176). Individual aldehydes and dicarbonyls reduce mesothelial cell proliferation, viability, and mediator synthesis in a dose dependent manner (177). Experimental data suggest the increased formation of AGE such as pentosidine and carboxymethyllysine (CML) in the presence of carbonyl compounds which is paralleled by the deposition of these AGE compounds in the mesothelial layer and the small vessel walls of the peritoneal membrane as demonstrated by immunohistochemistry (174). Moreover, reactive carbonyl compounds may be involved in the process of neoangiogenesis since methylglyoxal was found to induce the production of vascular endothelial growth factor (VEGF) in mesothelial cells (178).

**TABLE III - DOES SERUM PHOSPHATE (P) CONFORM TO THE CRITERIA FOR A UREMIC TOXIN?**

		Apply to P
1	The compound should be chemically identified and accurate quantitative analysis in biological fluids should be possible.	Yes
2	The plasma level of the compound should be higher in uremic than non-uremic patients.	Yes
3	High concentrations should be related to specific uremic symptoms that decrease or disappear when the concentration is reduced.	Yes for osteodystrophy, and almost always for vascular calcifications
4	When studying toxicity of specific compounds in human subjects, experimental animals and/or in appropriate <i>in vitro</i> systems, the concentrations used should be comparable to those found in the body fluids and/or tissue of uremic patients.	Yes

Systemically, carbonyl stress may also interfere with various cellular functions such as intra-cellular signalling and apoptosis. Moreover, AGE/ALE formation is believed to be involved in vascular injury observed in diabetes, atherosclerosis, diabetic nephropathy, and dialysis-related amyloidosis (171).

*h) Advanced oxidation protein products (BDL)*

Oxidative stress is defined as a disturbance in the prooxidant - antioxidant balance in favor of the former, resulting in the accumulation of oxidant-induced by-products of elementary components including DNA, proteins and lipids. Besides deleterious effects on biological compounds and signaling molecules, oxidants may also act as inflammatory mediators and this dual aspect of oxidative stress has to be equally considered in chronic renal failure (CRF).

Evidence of oxidative stress has been abundantly documented in CRF patients on maintenance hemodialysis (HD) in whom blood interaction with dialysis membranes triggers neutrophils to produce reactive oxygen species (ROS) (179). Additionally, uremia induces a severe defect in the activity of antioxidant systems and their cofactors. However this evidence has for a long time solely relied on the presence of increased circulating levels of lipid peroxidation products such as malondialdehyde or conjugated diene fatty acids, the relevance of which is still debated.

In the search for a more accurate marker, we recently described in the plasma of HD patients the presence of oxidized protein products which we designated "advanced oxidation protein products (AOPP)" in reference to advanced glycation end-products (AGE) with whom AOPP shared some homologies (180). As compared to controls, AOPP were found at high concentrations in the plasma of HD patients and were closely related to di-tyrosine, a marker of protein oxidation. *In vitro*, exposure of normal plasma or albumin to chlorinated oxidants such

as HOCl triggered the formation of AOPP that depended upon oxidant concentration. Size exclusion chromatography of uremic plasma allowed us to isolate high molecular weight AOPP (600 kDa) which are protein aggregates and low molecular weight compounds (around 80kDa), with albumin as a main component. However, in this study, high levels of AOPP were also found in both predialysis patients as well as in chronic ambulatory peritoneal dialysis-treated patients (180). In a following study we demonstrated that AOPP level increased with the progression of renal failure and was closely related to AGE and monocyte activation markers e.g., neopterin, TNF and its soluble receptors. Interestingly, *in vitro* preparations of AOPP were also able to trigger monocyte activation (181).

Taken together these findings suggested that AOPP are not only sensitive markers of oxidative stress but may also behave as potent mediators of inflammation (4).

The hypothesis that AOPP actively contribute to the uremic toxicity syndrome is in keeping with that of AGE in this setting. Indeed, the hypothesis that AGE proteins represent a novel class of the well-known toxic 'middle molecules' has long been proposed (5, 126). The recent demonstration by the group of Miyata and van Ypersele that oxidative processes contribute to the formation of AGE in uremia (182) also supports our proposal to consider AOPP as active uremic toxins.

Some proteins of crucial importance in dialysis-associated complications could be elective targets of AOPP formation.

Among these,  $\beta_2$ -microglobulin, involved in amyloid arthropathy, appears to be a good candidate, inasmuch as it represents an exquisite proteic target for glycation (183). Our previous observations showing that  $\beta_2$ -microglobulin can be fragmented and polymerized following exposure to superoxide anion and hydroxyl radicals also support this hypothesis (184). More recently, in collaboration with Argilès we also showed that following exposure to chlorinated oxidants,  $\beta_2$ -microglobulin undergoes extensive fibril formation (unpublished data).

Likewise, low density lipoproteins (LDL) involved in the accelerated development of atherosclerosis (185) might generate AOPP via oxidation of their protein moiety. Interestingly, a recent comparative study of the effects of LDL oxidized by HOCl or  $\text{CuSO}_4$  - which respectively affect the protein or the lipid moiety - on human macrophage cell lines has shown that LDL that had been treated by HOCl was much more potent in triggering the respiratory burst

**TABLE IV - ACCUMULATION OF REACTIVE CARBONYL COMPOUNDS IN UREMIA**

Compound	Reported concentrations (ng/ml)	
	Normal plasma	Uremic plasma
Glyoxal	67 ± 20	221 ± 28
Methylglyoxal	47 ± 12	110 ± 18
3-Deoxyglucosone	26 ± 16	59 ± 13

than its CuSO<sub>4</sub>-treated counterpart (186) but just as potent in inducing apoptosis of these cells (187).

Taken together, these findings suggest that AOPP may represent a novel class of uremic toxins and/or proinflammatory mediators and contribute to CRF-associated complications, notably accelerated atherogenesis.

### *i) Guanidino-compounds as uremic neurotoxins (PPDD)*

A series of guanidino compounds (GCs) have been suggested to be implemented in the uremic syndrome. Epileptic and cognitive symptomatology are manifestations of uremic encephalopathy.

For biochemical research in this area, cation-exchange chromatography and fluorescence detection are used (188). The determined guanidino compounds are  $\alpha$ -keto- $\delta$ -guanidinovaleric acid, guanidinosuccinic acid (GSA), creatine, guanidinoacetic acid,  $\alpha$ -N-acetylarginine, argininic acid,  $\beta$ -guanidinopropionic acid, creatinine (CTN),  $\gamma$ -guanidinobutyric acid, arginine, homoarginine, guanidine (G) and methylguanidine (MG). In addition, analytical work consists in the determination of the dimethylarginines (189).

GC levels have been determined in serum, urine, cerebrospinal fluid and brain of uremic patients (190, 191). Four GCs, namely CTN, G, GSA and MG, were highly increased, mainly as a consequence of reduced excretion and to a minor extent due to increased synthesis, and were subsequently called uremic GCs.

The behavioral research involved *in vivo* testing of toxicity and epileptic potency of uremic guanidino compounds after systemic or focal (intracerebroventricular and intrahippocampal) administration. The uremic GCs induced clonic-tonic convulsions in adult mice (192). GSA and MG were markedly more potent than G and CTN. Brain concentrations corresponding to intraperitoneal CD50 (convulsive dose in 50% of the animals) were 1328 nmol/g tissue for CTN, 209 nmol/g tissue for G, 56 nmol/g tissue for GSA, and 94 nmol/g tissue for MG. Brain CTN and GSA concentrations that induce clonic convulsions in mice are similar to concentrations found in brain of patients.

GSA appears to play an important role in the etiology of the hyperexcitability of the uremic brain. The compound induced clonic and tonic convulsions as well as epileptiform electrocorticographic discharges in adult mice (193). In addition, local administration in the brain by microdialysis resulted in visuospatial deficits.

Moreover, we set out to identify the possible mechanisms of action of these candidate uremic neurotoxins by performing electrophysiological experiments using intracellular registrations and patch-clamp methodology on primary dissociated mouse neurons in cell culture.

All four tested uremic guanidino compounds blocked  $\gamma$ -aminobutyric acid (GABA)- and glycine-evoked depolarization in mouse spinal cord neurons (194). GSA was shown to be the most potent compound. It was suggested that the uremic GCs might be blocking the GABA<sub>A</sub> and glycine receptor-associated chloride channel (194). Recent studies using the patch-clamp technique suggested that GSA, MG and CTN may rather act as competitive antagonists at the transmitter recognition site of the GABA<sub>A</sub> receptor (195). GSA, MG and CTN dose-dependently block the GABA-evoked inward as well as outward whole-cell currents (195). GSA was shown to be more potent than MG or CTN.

GSA-induced clonic convulsions were not or only slightly attenuated by antiepileptic drugs like diazepam or phenobarbital (193,196). Competitive and non-competitive *N*-methyl-D-aspartate (NMDA) receptor antagonists, on the other hand, effectively blocked these convulsions (196). Also, GSA potentiated NMDA- but not glutamate- or kainate-induced convulsions. These findings suggested that, in addition to the blockade of GABA-ergic inhibition, NMDA receptors were somehow involved in the GSA-induced convulsions. More recently, we have found behavioral and electrophysiological evidence that GSA acts as a selective agonist at NMDA-type excitatory amino acid receptors in a similar manner to the structurally related L-aspartate (197).

Based on the results summarized above, we proposed a hypothetical mechanism for the action of uremic GCs on glutamatergic transmission in the central nervous system (198): GSA evokes activation of NMDA receptors in conjunction with blockade of GABA<sub>A</sub> receptor ionophores; under these conditions, the pyramidal cells might be sufficiently depolarized to reduce the Mg<sup>2+</sup> block on NMDA receptors; activation of NMDA receptors elicits Ca<sup>2+</sup> influx, potentially causing calcium-mediated neurotoxicity; production of nitric oxide (NO) through calcium-dependent activation of NO synthase could be one of the mechanisms involved in sustained excitatory activity following GSA application.

Moreover, the joint presence of increased levels of uremic GCs could increase the block on GABA<sub>A</sub> receptors since co-application of G and MG results in a cumulative

inhibition of GABA responses (194). Moreover, GCs were shown to have other neurotoxic effects, which might also lead to neuronal depolarization.

Present research aims at expanding existing knowledge (199, 200) on the dialysis kinetics of the uremic guanidinocompounds and on the behavioral and electrophysiological effects of the above described and other guanidino compounds and polyamines.

#### *j) Homocysteine (ZAM)*

Homocysteine (Hcy) is a sulfur-containing amino acid that results from demethylation of dietary methionine. Reduced and oxidized forms of homocysteine are present in plasma, and their total fasting plasma levels (normally < 14  $\mu$ M), are thought to be a reflection of intracellular metabolism and cellular excretion of homocysteine (201).

Plasma total Hcy (tHcy) concentration increases in parallel with the degree of reduction in renal function, and persists after starting dialysis (201). The elevation of fasting tHcy concentrations is mainly due to the reduction of plasmatic Hcy clearance, although the cause of this decrease is still unknown (202). About 30% of the tHcy is not protein-bound and is filtered by the glomerulus. Urinary homocysteine excretion is negligible, however, since almost all filtered homocysteine is reabsorbed by tubular cells (203). Human renal metabolism of homocysteine measured in the fasting state also appears to be insignificant (204). A role of uremic-induced extrarenal defects in homocysteine metabolism remains plausible but not proven. An alteration of the remethylation pathway, but not of the transsulfuration pathway, could play a central role (205). The resistance to folate action observed in chronic renal failure patients may explain the alteration of the remethylation pathway, although the cause of this resistance is still unidentified (201). It is of note that folic acid therapy does not normalize tHcy clearance observed after homocysteine loading (202). Additional genetic factors and dialysis-related factors may also be involved (201).

Limited data suggests that the elevation of fasting circulating tHcy levels is associated with an increased risk of cardiovascular disease in chronic renal failure patients (201). The precise mechanism(s) underlying the atherogenic role of moderate hyperhomocyst(e)inemia in renal patients remain(s) to be determined. It has recently been suggested that the inhibition of the transmethylation

pathway, which is due to the decrease of S-adenosylhomocysteine-dependent methyltransferase activity, could be one possible link between hyperhomocyst(e)inemia and cardiovascular disease in chronic renal failure patients (206). On the other hand, moderate hyperhomocyst(e)inemia may predispose to cardiovascular disease through a mechanism that involves endothelial dysfunction and generation of reactive oxygen species (207). It should be noted, however, that long-term homocysteine-lowering treatment does not improve endothelial vasodilator function in chronic renal failure patients (208, 209). The presence of irreversible vascular damage in chronic renal failure patients could explain the lack of a beneficial effect of homocysteine-lowering treatment on endothelial function. On the other hand, the possibility that homocysteine is rather an indicator of an aberrant intracellular metabolism, or a player which exacerbates other cardiovascular risk factors, should still be considered.

Supplementation of folic acid at a pharmacological dose,  $\geq$  5mg/day, either alone or combined with vitamin B6 and B12, has been shown to reduce fasting plasma tHcy concentrations in chronic renal failure patients by about 30% (201). The role of active reduced forms of folic acid, which may lead to a greater decrease in fasting plasma total Hcy levels than those observed with folic acid supplementation in hemodialysis patients, is still under investigation (210-212). As of today, there are no conclusive data regarding the benefit of lowering fasting tHcy levels by folate supplementation on cardiovascular disease in chronic renal failure patients; based on this argumentation, folate supplementation cannot unequivocally be recommended (201). However, since this supplementation has no apparent side effects and is inexpensive, and since renal patients are considered patients with augmented cardiovascular risk, there is no formal objection to their therapeutic use either (201).

#### *k) Protein bound uremic solutes (RVH)*

Because of their strong protein binding, during dialysis most compounds of this group behave like middle molecules, in spite of low molecular weight. Removal by classical hemodialysis strategies, even with large pore membranes, is nevertheless disappointingly low (1).

Peritoneal dialysate contains larger quantities of protein bound compounds than hemodialysate (213), since peritoneal pore size allows the transfer of albumin together

with its bound moieties. Removal of entire albumin-ligand complex is at this moment probably the only way to remove sufficient amounts of protein bound compounds (213).

In what follows, prototypes of protein bound uremic solutes, such as p-cresol, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF), and indoxyl sulfate will be discussed. Homocysteine has been covered in the previous section (section 3).

P-cresol is a phenolic and volatile compound with a molecular weight of only 108.1 D. Its serum concentration is elevated in renal failure (214). P-cresol induces LDH-leakage from rat liver slices (215), inactivates the enzyme  $\beta$ -hydroxylase which plays a role in the transformation of dopamine to norepinephrine (216), and affects cellular oxygen uptake (217), drug protein binding (218), cell growth (219), and membrane permeability (220). P-cresol inhibits various metabolic processes related to the production of free radicals by activated phagocytes (221). Hepatocyte aluminum uptake and aluminum toxicity in hepatocytes are increased (2). P-cresol inhibits platelet-activating factor (PAF) synthesis (222), as well as arsenic detoxification by methylation (223).

P-cresol is an end-product of the metabolism of the amino acids tyrosine and phenylalanine by the intestinal bacteria (224). Prevention of the intestinal absorption of p-cresol by administration of oral sorbents decreases serum concentration in rats (225).

P-cresol is lipophilic and protein-bound, and its removal by hemodialysis is markedly lower than that of urea and creatinine (1). There is also no correlation between the removal of p-cresol and the removal of urea or creatinine (1).

3-Carboxy-4-methyl-5-propyl-2-furanpropionic acid (CMPF) is one of the urofuranic acids, a strongly lipophilic uremic solute, and one of the major inhibitors of the protein binding of drugs (226, 227) and of bilirubin (228). Its renal clearance is strongly reduced in renal failure (229). It inhibits the renal uptake of para-amino hippuric acid (PAH) in rat kidney cortical slices (230), and causes a decrease in renal excretion of various drugs. CMPF clearance in the rat is inhibited by PAH and probenecid (231). CMPF inhibits hepatic glutathione-S-transferase (232), deiodination of T4 and T3 by cultured hepatocytes (233) and ADP-stimulated oxidation of NADH-linked substrates in isolated mitochondria (234). Costigan et al demonstrated a correlation between plasma concentration of CMPF and neurologic abnormalities (235), and a

negative correlation with hemoglobin (236). CMPF inhibits erythropoiesis (237).

CMPF levels are lower in CAPD than in hemodialysis patients (237, 238). The strong protein binding of CMPF (1) hampers its removal during hemodialysis. Protein leaking hemodialysis induces a reduction of CMPF, and is at the same time related to a rise in hematocrit (239).

Indoxyl sulfate is metabolized by the liver from indole, which is produced by the intestinal flora as a metabolite of tryptophan. It enhances drug toxicity by competition with acidic drugs at the protein binding sites (240), inhibits the active tubular secretion of these compounds (241), and inhibits deiodination of thyroxine 4 (T4) by cultured hepatocytes (233). The oral administration of indole or indoxyl sulfate to uremic rats, causes a faster progression of glomerular sclerosis and of renal failure (242).

Because of protein binding, the intra-hemodialytic removal of indoxyl sulfate is far less important than that of other small compounds such as creatinine (3, 126, 233, 243, 244). Removal by CAPD is more effective (237).

Protein bound uremic solutes are contributing to a host of functional disturbances in uremia. At this moment no effective removal strategies are available, apart from peritoneal dialysis. As a consequence, it remains difficult to prove their patho-physiologic importance in a clinical setting. Adsorptive systems might offer a solution to this problem (245, 246).

#### 4) SPECIFIC PROBLEMS INVOLVED WITH CARDIOVASCULAR DAMAGE

Many of the solutes discussed in the previous sections, are involved, either directly or indirectly, in the induction of cardiovascular lesions. It is also well known that cardiovascular disease is more prominent in end stage renal failure (247, 248). In the following section, specific problems related to renal failure and cardio-vascular disease, but not directly to a specific toxin, will be discussed.

##### *a) Lowered lipoprotein lipase activity in uremic patients (BS)*

Lipoproteins constitute the main transport system for energy between organs through their carrying capacity for triglycerides and free fatty acids (FFA). Lipoprotein lipase

(LPL) will hydrolyse triglycerides transported by chylomicrons as well as very low density lipids (VLDL), thereby making FFA available for uptake by the tissues as metabolic fuel or for storage (249-254).

Uremic patients have an increased risk for cardiovascular diseases (255), and disturbances in lipid metabolism and transport play a role in this evolution.

In uremia, together with other disturbances in lipid metabolism, VLDL and intermediate density lipids are elevated, indicating a defect in the catabolism of the triglyceride-rich lipoproteins (256-260). This defect can be related to the decreased activity of LPL and hepatic lipase (259, 261, 262), since triglycerides (TG) are mainly hydrolysed by these enzymes (263).

LPL-activity is reduced in uremic animals (264), patients with chronic renal failure (265, 266) and patients on chronic hemodialysis (262).

Several factors may change LPL-activity, and some of these factors might be modified by the uremic status. In what follows we will discuss the patho-physiologic role of the main factors, together with the potential changes in activity which they may undergo during uremia.

LPL is produced in muscle and fat cells and then released extracellularly and displaced to the endothelial cell surface. There it interacts with heparan sulfate proteoglycans that cause a loose binding to the endothelial surface (267). One modulator of this system is albumin. FFA will inhibit the LPL activity in a feed back manner that will be counteracted by albumin by binding FFA (268). A negative effect could be present in those patients with decreased serum albumin (proteinuria, peritoneal dialysis, inflammatory processes and malnutrition).

LPL-activity is also reduced when insulin is increased. In uremic patients insulin levels are frequently increased due to insulin resistance (269-271).

Another modulator is the LPL-inhibiting factor (272, 273) found to be an apo-A-I-containing particle of pre- $\beta$ -electrophoretic mobility and minimal lipid content. The purified inhibitor contains apo-A-I as the only major protein. The inhibitory substance is more active in uremic subjects than in normals. Triglyceride concentration correlates both to inhibitor activity and degree of uremia. The lipase inhibitor most probably is an apo A-I/phospholipid octamer with a molecular weight of 230,000 Da (273).

Heparin is another factor with potential impact on LPL which is frequently applied in hemodialysis patients. Intravenous heparin will loosen LPL bound to heparan

sulphate at the endothelial surface. Thereby a part of the LPL binds to circulating lipoproteins and is transported to the liver where it is degraded (274).

In patients on chronic hemodialysis, the repeated administration of heparin each dialysis causes a repeated release of LPL from its binding sites, which eventually may result in a lack of LPL.

Finally, it has been suggested that the reduced lipase activity of uremia might be a result of secondary hyperparathyroidism (275, 276). However, those findings were not confirmed by others (277).

In conclusion, LPL-activity is reduced in uremic patients due to several possibly interacting factors. Future studies should focus on clarifying pathophysiological mechanisms of disturbed LPL-activity in uremic patients to enable prevention and specific therapies to halt cardiovascular complications and catabolic processes. Normalization of lipoprotein lipase and hepatic lipase activity by gemfibrozil results in the correction of lipoprotein abnormalities in chronic renal failure (278).

#### *b) Endothelial dysfunction in chronic renal failure (PB)*

One of the features of chronic renal failure (CRF) is endothelial dysfunction, with clinical implications such as atherosclerosis and susceptibility to infection. Uremic serum induces changes in the vascular subendothelium, characterized by a less intricate network of fibrils, a decreased attachment of endothelial cells (EC) and a reduced thrombogenicity of the extracellular matrix (279). Uremic serum inhibits nitric oxide synthesis in EC (280). Serum levels of endothelial glycoproteins, such as von Willebrand factor, tissue-type plasminogen activator (t-PA) and soluble thrombomodulin, which are thought to reflect endothelial dysfunction, are increased in uremia (281-283). In addition, the ability of the endothelium to release t-PA upon stimulation with desmopressin is reduced, possibly predisposing to thrombosis. A reduction of endothelium-dependent vasodilatation has also been demonstrated in uremia by studies using Doppler-ultrasound measurement of radial or brachial artery diameters (284) or forearm plethysmography (285). This defect may be one of the factors predisposing uremic patients to accelerated atherosclerosis. It has recently been shown that left ventricular hypertrophy and hypertrophy of the arterial wall are both related to the impairment of endothelial function, as estimated from determinations of the post-ischemic

capacity to vasodilate (286).

Numerous candidate uremic retention solutes, such as homocysteine, oxidation products, oxalic acid, advanced glycation end products (AGEs) and asymmetric dimethylarginine (ADMA) as an endogenous inhibitor of NO synthase (287), could be involved in uremic endothelial dysfunction.

Hyperhomocysteinemia has been associated with endothelial cell activation, as illustrated by an enhanced expression of adhesion molecules and tissue factor, as well as by leukocyte extravasation (288-290). Oxidation products are mainly responsible for the oxidation of LDL, which plays a role in the generation of foam cells, the initial lesions of atherosclerosis (291). In addition, oxidized LDL (ox-LDL) causes the alteration of various endothelial functions and activates the apoptosis of EC (292), inhibits EC migration (293), and delays endothelial wound healing (294). Ox-LDL reduces the EC expression of the antithrombotic glycoprotein thrombomodulin (295). Oxalic acid at uremic concentrations depresses EC replication and migration, functions which may be important for the constitutive inhibition of atherosclerosis (296). Advanced glycation end products (AGEs) that accumulate in CRF induce endothelial cell activation (297). AGE-albumin enhances the expression of EC adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1) and vascular cell-adhesion molecule-1 (VCAM-1) (297). AGE-albumin downregulates thrombomodulin and induces cell surface expression of procoagulant tissue factor. AGE-albumin increases endothelial permeability (298). AGEs also quench nitric oxide and thus interfere with endothelium-dependent vascular relaxation (299). P-cresol has recently been shown to decrease cytokine-induced expression of adhesion molecules and monocyte adhesion to cytokine stimulated EC (300). Since leukocyte adhesion to endothelial cells is a critical process in defense mechanisms, these effects could be involved in the immune function defect in uremic patients.

### *c) Pro-inflammatory cytokines (PS)*

Atherosclerotic cardiovascular disease (CVD) is the most common cause of death in ESRD-patients. The causes of the high prevalence of CVD in ESRD may be multifactorial. However, in view of the strong associations between atherosclerosis, malnutrition and inflammation (13) it may be speculated that, besides traditional risk factors, also factors associated with malnutrition and

inflammation may contribute to the excess prevalence of CVD. Indeed, inflammation, as evidenced by elevated CRP (301), cytokine (302,303) or soluble adhesion molecule levels (304), has been shown to predict cardiovascular and/or total mortality in ESRD patients. The prevalence of elevated CRP (>8-10 mg/l) in predialysis and dialysis patient populations is high and ranges between 30-50% (305). The causes of inflammation in ESRD patients are probably multifactorial. However, as the prevalence of inflammation is high already at the predialysis stage (13), it is evident that factors unrelated to the dialysis procedure *per se*, such as co-morbidity and decreased renal clearance of pro-inflammatory cytokines, contribute. All available evidence suggests that the pro-inflammatory cytokine system activity is elevated in ESRD patients (302) and several lines of evidence suggest that decreased renal clearance might play an important role. At first, strong positive correlations between GFR and various cytokines were found in a large cohort of undialyzed patients at various stages of ESRD (306). Second, Memoli et al (307) found significantly lower urinary IL-6R excretion in uremic patients compared to controls. Moreover, Poole et al (308) have found that the plasma half-life of IL-1 $\alpha$  was increased after nephrectomy and that the kidneys are the main organs through which IL-1 $\alpha$  is excreted. Finally, a strong linear correlation between the levels of TNF-receptors and renal function has been documented in ESRD patients (309). However, as the half-life of various cytokines is short and local tissue cytokine inactivation may be the most important pathway of cytokine degradation, more research is needed to determine the relative importance of the kidney in cytokine clearance. Nevertheless, as recent data has suggested that accumulation of TNF- $\alpha$  may contribute to the development of neurologic and hematologic complications in uremia, it has been suggested that TNF- $\alpha$  may, indeed, be considered a uremic toxin (310).

In conclusion, although it is evident that pro-inflammatory cytokines are elevated in ESRD patients, in part due to decreased renal clearance, it is not yet known whether elevated levels of cytokines are universal findings of uremia. Moreover, it has not yet been determined whether or not anti-cytokine therapies will reduce any specific uremic symptoms. Accordingly, on the basis of present knowledge, pro-inflammatory cytokines can be regarded as suspected but not as established uremic toxins (Tab. V).

## 5) TECHNICAL AND THERAPEUTIC ASPECTS

In 1980, the International Society of Artificial Organs organized a symposium on middle molecules in uremia to review analytical techniques, metabolic toxicity and clinical aspects. Bergström et al stated: “The research on uremic toxins is more than 150 years old, but the problem which compounds are responsible for the various symptoms of uremia remains to be solved.....” (311). Already at that time, a wide variety of fragments of known proteins developed specific signals and induced patho-physiologically relevant functional disturbances, and the different directions of research were notable from today’s perspective (inhibition of leukocyte function, neurotoxicity, kinetic modelling of middle molecules, selective treatment systems to enhance middle molecule clearance, effects on platelet aggregation, cardiotoxic effects, inhibition of cell growth).

When considering new initiatives on uremic toxicity in the continuously developing field of blood purification, some aspects deserve reflection.

### a) Technological perspectives (RD)

Essential equipment, devices and fluids for hemodialysis or peritoneal dialysis have become available for the removal of toxins by optimized diffusive and convective transport mechanisms: highly selective membranes with sharp cut-offs, non activating polymer materials, ultrapure dialysate and substitution fluids, and less toxic carbonyl free peritoneal dialysis fluids. There has been a trend over the last twenty years towards an improvement in the survival of patients on ESRD therapy, but it remains a challenge to show significant benefits

in prospective studies. Therefore, it is difficult to identify the key factors contributing to improved outcome in ESRD and to introduce new technologies. On the other hand, it is important to realize that progress in dialysis technology is required to cope with the increasing number of patients suffering from cardiovascular or infectious complications.

### b) Bioanalytical perspectives (RD)

Biochemical methods to identify structural and functional properties of biological substances has remarkably improved in the past decades, e.g. sophisticated HPLC-techniques, versatile immunological detection or structural analysis methods for proteins and peptides, molecular biology, advanced cell culture technology. They all help today to better predict the mode of action of uremic toxins and their specific role in complications associated with dialysis or uremia. Definition and functional characterisation of uremic toxins can certainly be extended and improved from today’s perspectives in the biotechnological “century”: e.g. specific knock-out animal models could help to better define and understand the role of specific substances in clinical complications.

Is it then sufficient to define and characterize substances as uremic toxins by new methodology or do we also have to widen the scope of uremic toxicity? In 1980, uremic toxicity was defined in relation to the uremic syndrome or to specific symptoms in end stage renal disease. As cardiovascular and immunological complications are today of major importance in the late phase of end stage renal disease by causing mortality and morbidity, it is certainly of importance to broaden the definition of uremic toxins to cover their potential role in severe ESRD associated complications.

**TABLE V - DO CYTOKINES CONFORM TO THE CRITERIA FOR UREMIC TOXINS?**

		Apply to cytokines
1	The compound should be chemically identified and accurate quantitative analysis in biological fluids should be possible.	Yes
2	The plasma level of the compound should be higher in uremic than non-uremic patients.	More studies are needed to determine this
3	High concentrations should be related to specific uremic symptoms that decrease or disappear when the concentration is reduced.	Not yet proven
4	When studying toxicity of specific compounds in human subjects, experimental animals and/or in appropriate <i>in vitro</i> systems, the concentrations used should be comparable to those found in the body fluids and/or tissue of uremic patients.	Yes

*c) Perspectives on improving correction of uremic toxicity (RD)*

Technologies to bio-engineer synthetic membranes - even combining them with other removal technologies - have greatly improved over the past 20 years. Based on existing platforms for membrane technology and processing blood compatible materials, it can be foreseen that “hybrid” devices consisting of synthetic materials compatible with biological structures, i.e. tissue engineered cells, or specific binding structures, might become technically achievable. However, technical innovation in treatment modalities needs to follow the constraints imposed by health care economics.

The following areas seem to be promising in designing the outline for new technologies:

- carbonyl stress compounds and Amadori-Maillard reaction products (AGE) (312,313)
- modulation of the complement system in uremia (314)
- nitric oxide system including Asymmetric Dimethylarginine (ADMA) (315)
- phosphate as key molecule of uremic toxicity (164)
- treatment modelling and simulation (316)

Progress in technology to correct uremic toxicity depends on improving outcome and quality of life in relation to therapy costs.

*d) Limitations of dialysis (UB)*

Although several uremic solutes are small enough to be efficiently removed by low-flux hemodialysis both from blood plasma and from other body compartments, hydrophilic middle molecular weight molecules in the range between 5 -12 KD can be better eliminated by convective techniques such as on-line hemodiafiltration (317) and hemofiltration (318, 319); these larger molecules may be too slow to diffuse out of their body reservoirs during intermittent dialysis. They rapidly re-appear in the plasma after dialysis. The dynamic balance between transfer rates across the dialyzer and the body's compartmental barriers, and between regeneration and metabolism, leads to the plasma concentration of a toxin. Very likely potential uremic toxins around and above 12 KD are poorly or incompletely removed by existing dialysis procedures. In addition, some low molecular weight protein-bound toxins are poorly removed due to their hydrophobic nature and their low uptake by a purely hydrophilic dialysate (1). Therefore, alternative removal strategies should be considered.

*e) Dialysis and adsorption (UB)*

There is renewed interest (320-322) in the concept of highly-specific or class-specific removal of potential uremic toxins by adsorption, in particular by membrane adsorption, as originally pioneered around 1970 (323). Adsorption, the process of toxins sticking to a surface, already occurs with all hemodialysis membranes, but solute removal is largely dependent on the amount of accessible surface area. For large solutes above 20 KD, only the luminal surface of 1 to 2 m<sup>2</sup> is freely accessible. Therefore, the adsorption capacity of dialyzers is small for such large toxins. Small toxins, on the other hand, which are transported across the inner membrane skin, are eliminated anyway by convection and diffusion: although they may be also adsorbed to the large inner porous surface of certain membrane materials, their clearance from the blood stream is hardly affected by this adsorption. Hence, additional sorbent devices and systems with much greater surface area (above 200 m<sup>2</sup>) and higher capacity for toxins of any size and hydrophobicity are developed or under clinical investigation.

Sorbent devices and techniques, used in combination with hemodialysis or hemodiafiltration, widely differ in concepts, complexity and performance. Sorbent techniques can be used to extract compounds from dialysate (e.g. hemodiadsorption (324), hemolipodialysis (325), albumin dialysis (326)), from ultrafiltrate (327,328) (in a regeneration procedure of ultrafiltrate before it is returned to the blood stream), from plasma (329,330) (if combined with plasma filtration strategies), or directly from the blood (331-334).

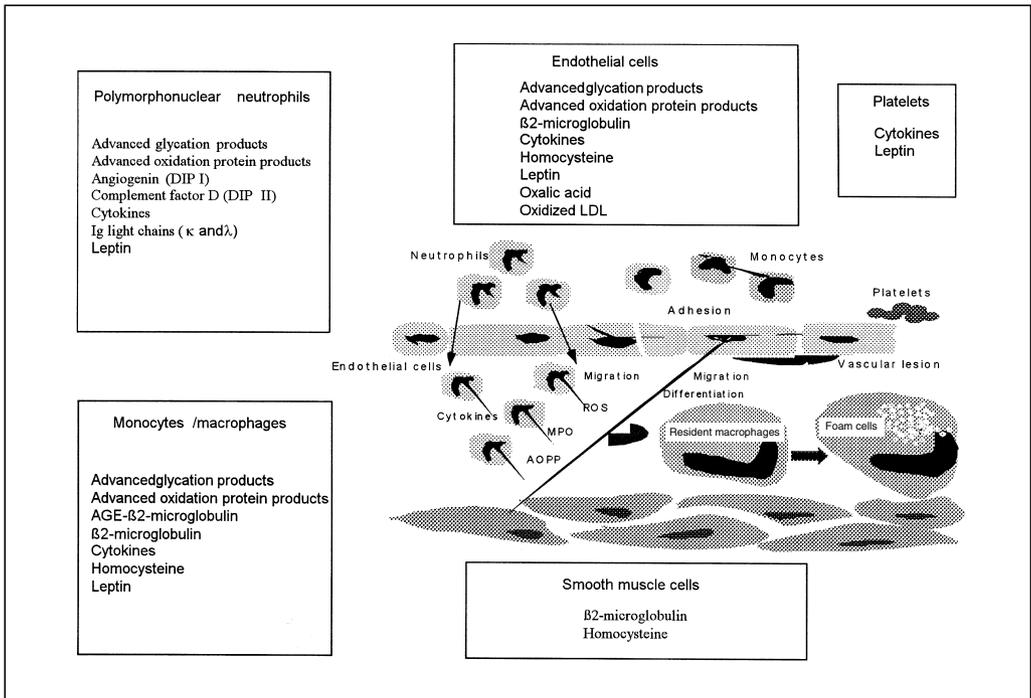
*f) New designs (UB)*

One of the new designs (335) of a high performance “direct hemoperfusion” device consists of a bundle of hollow fibres simply placed into a dialyzer-like housing. Blood enters the module and flows only along the outer walls of a bundle of hollow fibres, because the fibres are closed at the inlet side, but open at the outlet. The pressure drop drives the plasma through the microporous walls of the hollow fibres, while cellular material remains outside the microporous walls. As the plasma passes through the walls, it is exposed to immobilised ligands that bind the targeted components of the plasma. Then, at the down-stream end of the device,

**TABLE VI - TARGET FUNCTIONS INFLUENCED BY UREMIC TOXINS AND POTENTIALLY INVOLVED IN CARDIOVASCULAR AND CEREBROVASCULAR MORBIDITY**

Solute	Target cell	Modified function
Advanced glycation products	PMNL*	Activation glucose uptake (early products) Apoptosis (early products) Chemotaxis (late products)
	Endothelium	Cell activation Enhanced expression of adhesion molecules Downregulation thrombomodulin Induction cell surface expression of procoagulant tissue factor Increase permeability Quenching nitric oxide
Advanced oxidation protein products	Macrophages/ Monocytes	Apoptosis Activation of NADPH-oxidase and myeloperoxidase dependent oxygenation activities Increased synthesis of TNF- $\alpha$
AGE- $\beta$ 2-microglobulin	Macrophages/ Monocytes	Recruitment Delayed apoptosis Increased synthesis of TNF- $\alpha$ , IL-1 and IL-6
Angiogenin (DIP I)	PMNL*	Degranulation
Asymmetric dimethylarginine (ADMA)	Endothelium	Endogenous inhibition NO-synthase Cell proliferation
$\beta$ 2-microglobulin	Macrophages/ Monocytes	Decreased binding of $\alpha$ 2-microglobulin Lysosomal accumulation and monocyte death Increased adhesion
Complement factor D (DIP II)	SMC*	Contraction
	PMNL*	Degranulation
Cytokines	Thrombocytes	Stimulation thrombogenesis
	Macrophages/ Monocytes	Activation Induction tissue factor expression Release serine proteases
	Endothelium	Endothelial dysfunction Induction tissue factor expression Upregulation adhesion molecules Production vasoactive agents Induction procoagulant activities Changes fibrinolysis
Ig light chains ( $\kappa$ and $\lambda$ )	PMNL	Chemotaxis Activation of glucose uptake Activation of basal oxidative metabolism
Homocysteine	Endothelium	Cell activation Enhanced expression adhesion molecules Enhanced secretion of cytokines Enhanced expression tissue factor Leukocyte extravasation Induction apoptosis
Leptin	Macrophages/ Monocytes	Stimulation NF $\kappa$ -B Increased cytokine expression
	Thrombocytes	Promotion aggregation
	Macrophages/ Monocytes	Increase LPS*-induced cytokine production Inhibition monocyte-mediated response Enhancement phagocytic activity Induction oxidative stress
	Endothelium	Enhancement proliferation Induction expression TGF $\beta$ -1
Oxalic acid	Endothelium	Depression cell migration and replication
Oxidized LDL	Endothelium	Inhibition cell migration Delay wound healing

\*: PMNL - polymorphonuclear leukocytes; SMC - smooth muscle cells; LPS - lipopolysaccharide; TGF: Tissue Growth Factor



**Fig. 3 - Uremic solutes with known negative impact on the cell systems involved in atherogenesis and the clinical development of cardiovascular and cerebrovascular problems (present state of knowledge).**

where the hollow fibres are open, the plasma flow recombines with the blood flow before exiting the module, so that whole blood, minus the adsorbed ligand, is returned to the patient.

**6) GENERAL CONCLUSIONS (RV)**

Exciting new paths lay ahead in the field of uremic toxicity. Progressively more and more compounds are being recognized, that could play a role in the many biochemical, biological and clinical changes that affect the uremic patient. Our knowledge of pathophysiologic events, especially those involved in the development of cardiovascular and immunologic morbidity, should be extended. Based on this additional knowledge, applied research should be ready to supply new devices and strategies, as well as drugs, able to correct or prevent these patho-physiologic alterations.

A specific area of concern is the vascular damage induced by a number of toxins. Overall, a number of solutes, retained in uremia, might specifically interfere with cellular functions involved in pro-inflammatory mechanisms and atherogenesis. The most important solutes involved in this process are set out in Figure 3, together with their target cells. In Table VI, their specific modes of action are

listed. It should be stressed that the present status of knowledge is illustrated here and that a systematic approach to this problem might further unravel the presence of more compounds with an impact in this area. Decreasing the retention of responsible solutes to reduce cardio-vascular morbidity which has a substantial weight on the outcome in the uremic and/or dialyzed population should be considered.

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