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UREMIC TOXINS IN CHRONIC RENAL FAILURE

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A b s t r a c t: The uremic syndrome is a complex mixture of organ dysfunctions, which is attributed to the retention of a myriad of compounds that under normal conditions are excreted by healthy kidneys. During recent years major steps have been taken in the area of identification and characterization of uremic retention solutes and in the knowledge of their pathophysiological importance; however, our knowledge remains far from complete. In the present paper the general classification based on their molecular weight and on their protein-binding characteristics, with reflections on their removal, will be discussed. In addition, current knowlegde about the main uremic retention products and their clinical and biological effects will be reviewed in detail.

Key words: uremic toxins, chronic renal failure, dialysis.

Introduction

The uremic syndrome is a complex of biological and biochemical alterations that result in a host of failing organs and disturbing symptoms. It originnates from the retention of solutes that under normal conditions are cleared by the kidneys into the normal urine, although derangements of hormonal, metabolic and enzymatic axes also play a role. The impact of retention is underscored by the clinical improvement resulting from dialysis and kidney transplantation.

The uremic syndrome is characterised by a deterioration of biochemical and physiological functions (Table 1) in parallel with the progression of renal failure. This results in a variable number of symptoms, which mimic the picture

of exogenous poisoning. Although the link between clinical deterioration and uremia had already been recognised decades ago, and although the number of new patho-physiological elements provided in this area has risen exponentially over the last few years, our knowledge about the responsible factors remains incomplete.

Current knowledge concerning uremic solute retention and its clinical and biological effects will be reviewed below.

Table 1 – Табела 1

The uremic syndrome – main clinical alterations
Уремичен синдром – главни клинички алтерации

Cardiovascular system
atheromatosis
arteriosclerosis
cardiomyopathy
decreased diastolic compliance
hyper/hypotension
pericarditis

Nervous system
concentration disturbances
cramps
dementia
depression
fatigue
headache
motor weakness
polyneuritis
reduced sociability
restless legs
sleep disorders
stupor,coma

Hematological system / coagulation
anemia
bleeding
hypercoagulability

Immunological system
inadequate antibody formation
stimulataion of inflammation (baseline)
susceptibility to cancer
susceptibility to infection

Endocrinology
dyslipidemia

glucose intolerance
growth retardation
hyperparathyroidism
hypogonadism
impotence, diminished libido

Bone disease
adynamic bone disease
amyloidosis ($ß_2$-microglobulin)
defective calcitriol metabolism
osteitis fibrosa
osteomalacia
osteoporosis

Skin
melanosis
pruritus
uremic frost

Gastro-intestinal system
anorexia
dyspepsia
gastro-intestinal ulcers
hiccup
nausea, vomiting
pancreatitis

Pulmonary system
pleuritis
pulmonary edema
sleep apnoea syndrome

Miscellaneous
hypothermia
thirst
uremic foetor
weight loss

UREMIC SOLUTE RETENTION

A) *General Classification of the Uremic Solutes*

A gradual retention of a large number of organic metabolites of proteins, fatty acids and carbohydrates characterises the progression of renal failure, whereby partial metabolization and elimination by other than renal pathways may compensate for the loss of renal clearance. Some of the retained compounds are proven toxins. Toxicity is not a simple monofactorial process whereby only one or a few toxins affect many different metabolic processes at a time. Other retained substances are non-toxic but can be used as markers of retention.

A recent survey of the literature revealed the retention in uremia of at least 90 compounds, of which the concentration had been reported [1]. It is very likely that this is only the tip of the iceberg. Whereas in the survey mentioned above approximately 25 middle molecular weight peptides were described, a recent study by highly sophisticated proteome analysis revealed the presence of at least 1000 such compounds in ultrafiltrate from dialysed patients [2].

Under normal conditions, the glomerular filter clears molecules with a molecular weight up to ± 58,000 Dalton. It is supposed that all these substances are retained in renal failure. An additional role should be attributed to changes in tubular secretion, reabsorption and metabolic breakdown, which are all altered when renal mass decreases. The molecules metabolized by the kidneys may have a higher molecular weight (> 58,000 D) than those cleared. Renal and non-renal metabolization of solutes and non-renal clearance may in their turn be inhibited following uremic retention.

Uremic retention products are arbitrarily subdivided according to their molecular weight [3, 4]. Low molecular weight molecules are characterised by a molecular weight (MW) up to 500 D [e.g. urea (MW: 60), creatinine (MW: 113)]. They can further be subdivided iton protein-bound and non-protein-bound molecules. Substances with a molecular weight range above 500 D are called middle molecules [e.g. parathyroid hormone (PTH, MW: 9,424), β_2-microglobulin (β_2-M, MW: 11,818)]. Several clinical, metabolic and/or biochemical disturbances such as food intake, apolipoprotein (apo) A-I secretion, osteoblast mitogenesis, cell growth, lymphocyte proliferation and interleukin production are caused by uremic compounds that conform to the middle molecular weight range [5-10]. Several of the recently defined uremic compounds, e.g. $ß_2$-M, various peptides, some of the AGE, as well as PTH,-conform to the definition of the middle molecules (MM) (see below).

Dialysis membranes with the capacity to remove MM (high flux membranes) have been related to lower mortality [11–15], as well as a slower loss of residual renal function [16], less preponderant dyslipidemia [17], improvement of polyneuropathy [18] and a lower prevalence of the carpal tunnel syndrome [19]. However, these highly efficient membranes are often at the same time less complement activating than unmodified cellulose, in many studies their counterpart. Hence, the relative importance of the removal of MM versus biocompatibility-related events is not always clear. Two studies, however, point to an independent benefit of large molecule removal [20, 21].

In the prospective randomized HEMO-study, however, no significant impact on mortality was found for high-flux dialyzers upon primary analysis, although there was a trend [22]. Upon secondary analysis a benefit was found for large pore membranes regarding cardio-vascular events [22]. Patients who had been treated for a long time on dialysis profited of an extra benefit [23].

Removal of larger molecules is more efficient when the high flux membranes are used in a convective mode [24]; no data is available whether this affects mortality. Convective treatment modalities have a positive impact on the development of the carpal tunnel syndrome [19]. On-line hemodiafiltration with large convective volumes results in a rise of erythrocyte counts and a decrease of erythropoietin needs [25]. Even if highly efficient dialysis is clinically superior, its cost-effectiveness still needs to be demonstrated.

Small protein-bound compounds such as hippuric acid or indoxyl sulphate behave like MM during dialysis, due to their high protein binding. Their removal by classical hemodialysis systems, even with large pore membranes, remains disappointingly low [26], which may be attributed to the complex distribution and intra-dialytic kinetics of these compounds. Therefore, alternative removal strategies other than the classical ones should be considered, such as adsorption, changes in timeframes, use of protein-leaking membranes and/or stimulation of metabolic pathways. Even small water-soluble compounds, which in principle should show the same characteristics as urea, quite often show different kinetics, as has been demonstrated recently by the guanidines [27].

Peritoneal dialysate is a much richer source of protein-bound compounds than hemodialysate [28], since peritoneal pore size allows the transfer of substantial quantities of albumin together with its bound moieties, which is not the case for even the most open hemodialyzer membranes. Also the continuous timeframe might enhance the removal of these compounds [29].

Until recently, no data had confirmed a potential clinical impact of protein-bound molecules. Recently, at least two studies have pointed in that direction [30, 31].

B) *Main uremic retention products*

Several uremic retention solutes influence biological functions. Other compounds have no proven direct toxicity, but may be useful markers of uremic retention. An overview of the pathologically most relevant uremic retention solutes with their molecular weight is given in table 2. It should be acknowledged that anorganic compounds such as water and potassium exert toxicity as well. In what follows, we will concentrate on the organic retention compounds.

1) Advanced glycation end-products (AGE)

As first described by Maillard, glucose and other reducing sugars react nonenzymatically with free amino groups to form reversible Schiff base adducts (in days) and stable Amadori products (in weeks), which are then converted into AGE through chemical rearrangements and degradation reactions [32]. Several AGE-compounds are peptide-linked degradation products [33] (MW 2,000-6,000 D), although the baseline AGE-products such as pentosidine, 2-(2-fuoryl)-

4(5)-(2-furanyl)-1H-imidazole (FFI), imidazolone, 3-deoxyglucosone, pyrrole aldehyde, and N^{ε}–(carboxymethyl)lysine have a substantially lower MW (Table 2).

Table 2 – Табела 2

Major uremic retention solutes and their molecular weight (Daltons)
Најважни ретенциони уремични соединенија и нивните молекуларни тежини (Далтони)

Compound	MW	Compound	MW
ADMA/SDMA	202	Adrenomedullin	5729
ANF	3080	Benzylalcohol	108
ß-endorphin	3465	ß-guanidinopropionic acid	131
ß2-microglobulin	11818	CGRP	3789
Cholecystokinin	3866	CIP	8500
Clara cell protein	15800	CML	188
CMPF	240	Complement factor D	23750
Creatine	131	Creatinine	113
Cystatin C	13300	Cytidine	234
DIP I	14400	DIP II	24000
3-deoxyglucosone	162	Dimethylarginine	202
Endothelin	4283	γ-guanidinobutyric acid	145
Glomerulopressin	500	GIP I	28000
GIP II	25000	Guanidine	59
Guanidinoacetic acid	117	Guanidinosuccinic acid	175
Hippuric acid	179	Homoarginine	188
Homocysteine	135	Hyaluronic acid	25000
Hypoxanthine	136	Imidazolone	203
Indole-3-acetic acid	175	Indoxyl sulfate	251
Leptin	16000	Melatonin	126
Methylguanidine	73	Myoinositol	180
Neuropeptide Y	4272	Orotic acid	156
Orotidine	288	o-OH-hippuric acid	195
Oxalate	90	p-cresol	108
p-OH-hippuric acid	195	Parathyroid hormone	9225
Pentosidine	135	Phenylacetylglutamine	264
Phenol	94	Phosphate	96
Pseudouridine	244	Putrescine	88
Retinol binding protein	21200	Spermine	202
Spermidine	145	Thymine	126
Trichloromethane	119	Tryptophan	202
Urea	60	Uric acid	168
Uridine	244	Xanthine	152

AGE are retained not only in renal failure, but also in diabetes mellitus and ageing [34], where they are held responsible for tissular damage and functional disturbances. In the uremic population, the level of glucose-modified proteins is higher than in diabetics without renal failure [35], and AGE-concentration does not depend on the glycemic status [36, 37]. The production of AGE in ESRD has been related to oxidative and carbonyl stress, rather than to reactions with glucose [38]. Not all AGE-generation is oxidative, however. AGE provoke monocyte activation [39], as well as the induction of interleukin-6, tumour necrosis factor-α, and interferon-γ generation [40]. AGE-modified $ß_2$-M may play a role in the generation of dialysis-associated amyloidosis [41] (see below). Serum pentosidine levels are higher in patients with dialysis-related amyloidosis, compared to their amyloid-free counterpart [42]. AGE can react with and chemically inactivate nitric oxide (NO) [43], a potent endothelium-derived vasodilator, anti-aggregant and antiproliferative factor. AGE are also related to oxidative protein modification [44]. 3-Deoxyglucosone inactivates glutathione peroxidase, a key enzyme in the neutralisation of hydrogen peroxide [45]. AGE accumulate in atheromatous plaque of the aortic wall of subjects with ESRD, where they may contribute to a more rapid progression of atherosclerosis [42]. To our knowledge, there is however no observational study of uremia, linking AGE directly to atherogenesis.

Late glycation products increase PMNL chemotaxis [46]. Other recent data suggest that whereas AGE increase baseline leukocyte response, activated response to infectious stimuli is blunted [47]. This suggests a dual response, related at the clinical level both to atherogenesis and susceptibility to infection [48].

Most of the biological actions of AGE that have been registered up to now have, however, not been obtained with AGE recovered from uremic or diabetic serum, but with AGE artificially prepared in the laboratory [48]. Recent data underscore as well the immune enhancing effect of genuine AGE, as they are found in renal failure [49].

Concentrations in ESRD patients might be attributed to increased uptake, production and/or retention. During industrial food processing, cooking procedures and storage of foods, food proteins are modified by carbohydrates [50–52], and those are absorbed via the gastro-intestinal tract [36]. Healthy kidneys are responsible not only for glomerular filtration but also for tubular reabsorption and degradation of AGE [53, 54]. Specific receptors for AGE have been identified (RAGE) and their expression is enhanced during uremia [55]. AGE binding to RAGE has been shown to stimulate mesothelial cell activity, and results in overexpression of vascular cell adhesion molecule (VCAM-1), which activates human peritoneal cells and promotes local inflammation, implicating the development of tubular injury [56].

In spite of continuous contact with glucose via the dialysate, CAPD patients do not have higher serum AGE levels than hemodialysis patients [33]. Nevertheless, protein glycation has been demonstrated in the peritoneal membrane [57]. The heat sterilisation of glucose-containing peritoneal dialysate induces the formation of glucose degradation products (GDP), which are precursors of AGE [58]. GDP inhibit leukocyte response, and this effect is attenuated when heat sterilization is replaced by other procedures (e.g. filter sterilization) [59].

Removal of AGE is significantly more important with high flux hemodialysis than with conventional dialysis with low flux membranes [60]. AGE show a marked heterogeneity in removal pattern, even during high flux dialysis [51, 60]. It is unclear which compounds could be representative by their removal pattern in such a way that they could serve as a marker for the overall group of AGE.

2) $ß_2$-microglobulin ($ß_2$-M)

$ß_2$-M (MW approximately 12,000D) is a component of the major histocompatibility antigen. Uremia-related amyloid is to a large extent composed of $ß_2$-M, and is essentially found in the osteo-articular system and in the carpal tunnel, although deposition can be systemic as well [62]. Uremia-related amyloidosis becomes most often clinically apparent after several years of chronic renal failure and/or in the aged [63]. According to the most recent studies, its prevalence tends to decrease [64], probably due to modifications in dialysis strategies.

AGE-modified $ß_2$-M has been identified in amyloid of hemodialysed patients [65] and enhances monocytic migration and cytokine secretion [66], suggesting that foci containing AGE-$ß_2$-M may initiate inflammatory response, leading to bone and joint destruction. The lack of a higher clinical incidence of $ß_2$-M-amyloidosis in diabetic dialysis patients [67], who generate large quantities of AGE in the presence of hyperglycemia, casts a doubt on the pathophysiologic role of AGE in amyloid formation. Possibly, the AGE-transformation plays a more important role in the inflammation surrounding $ß_2$-M-amyloid than in its generation.

Long-term hemodialysis with large pore membranes results in a progressive decrease of pre-dialysis $ß_2$-M concentrations; the levels remain, however, far above normal, even after intensive removal therapy [68, 69]. Long-term dialysis with large-pore dialyzers results in a lower prevalence of dialysis-related amyloidosis and/or carpal tunnel syndrome [11, 19, 70, 71]. Whether this benefit is attributable to a better removal of $ß_2$-M, to lower complement and leukocyte activating capacity, or to protection against the transfer of dialysate impurities into the blood stream (e.g. lipopolysaccharides) [64] is not evident,

since most of the dialyzers associated with a lower incidence of amyloidosis have all three abovementioned properties.

Because $ß_2$-M is only removed by dialyzers with a large pore size, its kinetic behaviour might be representative of other large molecules. The behaviour of $ß_2$-M during dialysis is, however, not necessarily representative of that of other MM. Discrepancies in behavior in the long run have been demonstrated in relation to other MM, such as complement factor D [72].

Recently, several devices with strong adsorptive capacity for $ß_2$-M have been developed [73].

The clinical expression of dialysis-related amyloidosis disappears after kidney transplantation, but the underlying pathological processes such as bone cysts and tissular $ß_2$-M remain preserved [74]. Possibly, immunosuppressive therapy plays a role in the regression of the symptomatology.

Serum creatinine concentration is not only the resultant of uremic retention but also of muscular break-down; therefore, a high serum creatinine may be the consequence of high muscular mass, and hence an indicator of metabolic well-being. Morbidity and mortality in hemodialysed patients are positively correlated with serum creatinine [75].

3) Cytokines

In view of the strong associations between atherosclerosis, malnutrition and inflammation [76], it may be speculated that factors associated with malnutrition and inflammation may contribute to the excess prevalence of cardiovascular disease. The causes of inflammation in ESRD patients are probably multifactorial. All available evidence suggests that the pro-inflammatory cytokine system activity is elevated in ESRD patients [77]. It has been hypothesized that epoetin resistance is due to enhanced levels of immune activation since chronic inflammation can modify the process of erythropoiesis. The accumulation of TNF-α may contribute to the development of neurologic and hematological complications in uremia. Several lines of evidence suggest that decreeased renal clearance might play an important role [78]. 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.

4) Dinucleoside polyphosphates

Dinucleoside polyphosphates are a group of substances described to be involved in the direct regulation of the vascular tone as well as growth of vascular smooth muscle cells [79] and mesangial cells [80]. Specific members of this group, the diadenosine polyphosphates, have been detected in hepatocytes, human plasma [81] and platelets. In addition, concentrations of diadeno-

sine polyphosphates were shown to be increased in platelets [82] from hemodialysis patients [83]. Recently, uridine adenosine tetraphosphate (Up4A) was isolated and identified as a novel endothelium-derived vasoconstrictive factor. Its vasoconstrictive effects, its plasma concentration and its release upon endothelial stimulation strongly suggest that Up4A has a functional vasoregulatory role [84].

5) Guanidines

Guanidines are structural metabolites of arginine. Among them are well known uremic retention solutes, such as creatinine and guanidine, and newly detected moieties such as asymmetric and symmetric dimethylarginine (ADMA and SDMA).

Guanidine levels have been determined in serum, urine, cerebrospinal fluid and brains of uremic patients [85, 86]. Four compounds, creatinine, guanidine, guanidinosuccinic acid (GSA) and methylguanidine (MG) are highly increased.

Several of the guanidino compounds modify key biological functions. GSA inhibits the production by 1α-hydroxylase of the active vitamin D metabolite, $1,25(OH)_2VitD_3$ (calcitriol) [87], and interferes with activation of ADP-induced platelet factor 3 [88] at concentrations currently found in hemodialysed uremics [89, 90]. A mixture of guanidino compounds suppresses the natural killer-cell response to interleukin-2 [91] and free radical production by neutrophils [92]. In recent studies, guanidine compounds have been shown to enhance baseline immune function, related to vascular damage [93]. In addition, they also have been related to a decreased protein binding of homocysteine, another compound with vessel-damaging potential [94].

GSA, γ-guanidinobutyric acid, methylguanidine, homoarginine and creatine induce seizures after systemic and/or cerebroventricular administration to animals [95, 96]. GSA plays an important role in the hyperexcitability of the uremic brain [97]. GSA probably also acts as a selective agonist at the N-methyl-D-aspartate (NMDA) receptor [98, 99]. GSA displays in vivo and in vitro neuroexcitatory effects that are mediated by ligand- and voltage-gated Ca^{2+} channels, suggesting an involvement of the guanidines in the central nervous complications of uremia [100].

Arginine enhances NO-production. Some of the other guanidines, as arginine-analogues, are strong inhibitors of NO-synthase. The inhibition of NO-synthesis results in saphenous [101] and mesenteric vasoconstriction [102], hypertension [103], ischemic glomerular injury [104], immune dysfunction [105] and neurological changes [106]. ADMA is the most specific endogenous compound which inhibits NO-synthesis. ADMA accumulates in the body during the development of renal failure [107, 108], related to decreased renal excretion but possibly also to suppressed enzymatic degradation by dimethylarginine dime-

thylaminohydrolase [109]. The increase in SDMA is more pronounced, but this compound is biologically less active. In the brain, ADMA causes vasoconstriction and inhibition of acetylcholine-induced vasorelaxation [110]. Also in thoracic and radial vessels, ADMA induces contractions [111]. Recently, estrogen has been shown to alter the metabolism of ADMA reducing the circulating concentration in vivo [112]. Methylguanidine, another endogenous guanidine, also shows a certain inhibitory activity on cytokine- and endotoxin-inducible NO-synthesis, albeit to a limited extent [113].

In contradiction to the hypothesis of inhibition of NO-synthesis in uremia, Noris *et al.* described an enhanced NO-production, in patients susceptible to uremic bleeding tendency [114]. Possibly, this effect is limited to a subgroup of the uremic population.

In the renal proximal convoluted tubule of rats with renal failure, the generation out of arginine of guanidinoacetic acid and creatine is depressed [115], whereas the synthesis of GSA, guanidine and methylguanidine is markedly increased, due to urea recycling.

Dialytic removal of guanidino compounds is subjected to a substantial variability [90]. Possibly, tissular distribution or protein binding play a role. In spite of a low MW, removal by hemodialysis of ADMA is only in the range of 20-30% [108]. Several of the guanidines have a substantially larger distribution volume than the standard marker urea, resulting in a decreased dialytic effective removal and substantial post-dialysis rebound [27].

6) Homocysteine

Homocysteine (Hcy), a sulphur-containing amino acid, is produced by the demethylation of dietary methionine. Retention results in the cellular accumulation of S-adenosyl homocysteine (AdoHcy), an extremely toxic compound, which competes with S-adenosyl-methionine (AdoMet) and inhibits methyltransferase [116]. Moderate hyperhomocysteinemia, caused by a heterozygous deficiency of Hcy breakdown or by vitamin B_6, B_{12} or folate deficiency, is an independent risk factor for cardiovascular disease in the general population [117, 118]. Reduced and oxidized forms of Hcy are present in the plasma, and total fasting levels are a reflection of intracellular metabolism and cellular excretion of Hcy [119].

Hcy increases the proliferation of vascular smooth muscle cells, one of the most prominent hallmarks of atherosclerosis [120]. Moderate hyperhomocysteinemia may involve endothelial dysfunction and generate reactive oxygen species [121]. The administration of excessive quantities of the Hcy precursor methionine to rats induces atherosclerosis-like alterations in the aorta [122]. Hcy also disrupts several anticoagulant functions in the vessel wall, which results in enhanced thrombogenicity [123]. Guanidines have been related to re-

lease of homocysteine from its protein-binding sites, by induction of structural modifications of albumin [94].

Patients with chronic renal failure have total serum Hcy levels two- to fourfold above normal. The serum concentration depends not only on the degree of kidney failure, but also on nutritional intake (e.g. of methionine) [124], vitamin status (e.g. of folate) [125, 126], genetic factors [127–129] and decreased renal metabolization [116]. Almost all filtered Hcy is reabsorbed in the tubular system so that urinary excretion is minimal [130]. Detoxification by remethylation of homocysteine to methionine is inhibited in hemodialysis patients [131, 132].

Hyperhomocysteinemia is the most prevalent cardiovascular risk factor in ESRD [129, 133]. Plasma homocysteine and cardiac mass correlate to each other [134]. In a study by Suliman *et al.*, however, total plasma Hcy was lower in hemodialysis patients with cardiovascular disease than in those without [124]. In this study, a correlation was found between total Hcy and serum albumin, pointing to a negative impact of malnutrition on Hcy concentrations. Also more recent data point to an inverse relation between homocysteine levels and mortality [135]. Hcy is partly bound to albumin, which hampers removal by hemodialysis. Hyperhomocysteinemia is more pronounced in hemodialysis patients, than in PD [126]. In hemodialysed patients, homocysteine levels correlate with plasma folate [125, 126], and with the activity of enzymes that are at play in Hcy-metabolism. Even with peritoneal dialysis, it is impossible to reduce total Hcy plasma levels to normal [136].

Dialysis with extremely leaky hemodialyzer membranes with large pore size (so-called super-flux membranes) results in a progressive decline of predialysis plasma homocysteine concentrations [137]. This effect has at least in part been attributed to changes in homocysteine metabolism, induced by enhanced middle molecule removal through these highly efficient membranes.

Hcy levels can be reduced by folic acid, vitamin B_6 and vitamin B_{12} [138]. The population with ESRD might require high quantities of vitamins [139].

Possibly, the disappointing efficiency of folic acid might be related to an impairment of the metabolization of folic acid to 5-methyltetrahydrofolate (MTHF), which is the active compound in the remethylation pathway [140]. In an attempt to obviate such a deficiency, Bostom *et al.* directly administered oral MTHF (17 mg/d) to hemodialysed patients [141]. No benefit was found, however. Touam *et al.*, on the other hand, could reduce total Hcy to normal in approximately 80% of the studied population, by the administration of folinic acid, a precursor of MTHF [140]. Since the supplementation with folate is inexpensive and relatively harmless, there is no formal objection to it's therapeutic use.

Direct clinical proof of the benefit of a lower Hcy concentration in uremia is to our knowledge not available. Even when it was possible to decrease Hcy levels therapeutically, carotid artery stiffness was not altered [142].

7) Indoxyl sulphate

Indoxyl sulphate 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 [143], inhibits the active tubular secretion of these compounds [144], and inhibits deiodination of thyroxin 4 by cultured hepatocytes [145].

The oral administration of indole or indoxyl sulphate to uremic rats causes a faster progression of glomerular sclerosis and of renal failure [146]. This effect is possibly mediated by the renal gene expression of transforming growth factor ß (TGFß), tissue inhibitor of metalloproteinase-1 (TIMP-1) and pro-alpha1(I)collagen [147]. In animals, progression of renal failure is refrained by adsorbant administration, together with a diminished expression of the above-mentioned factors [147]. Indoxyl sulphate refrains endothelial repair upon trauma [148].

Reduction of serum indoxyl sulfate concentration, by intra-intestinal absorption of the precursor indole, reduces uremic itching [149]. AST-120 retards the development of acquired renal cystic disease and aortic calcification [150], and ameliorates tubulo-interstitial injury by reducing the expression in the kidneys of ICAM-1, osteopontin, TGF-beta1 and clusterin in uninephrectomized rats [151].

Because of protein binding (approximately 100% in normal subjects and 90% in uremics), the intra-dialytic behaviour of indoxyl sulphate diverges from that of other small compounds such as creatinine. Removal by CAPD is more effective [152]. High-flux hemodialysis does not enhance removal [26]. Alternative extracorporeal removal procedures such as hemoperfusion might be considered. Dialysis against albumin-containing dialysate removes albumin-bound uremic toxins such as indoxyl sulphate more efficiently than conventional dialysis and may be useful for reducing these compounds [153].

8) Oxidation products

Oxidative capacity is increased in uremia [154–156] both before and after the start of dialysis [44]. Uremic patients also show an impaired antioxidant response, partly related to plasma glutathione deficiency [157].

Oxidatively modified proteins act as mediators of oxidative stress and monocyte respiratory burst [44]. Albumin seems to be one of the target proteins of these oxidative reactions [44, 158]. Structural modification of albumin may alter its binding capacity for drugs and other solutes [159]. Modification of

hemoglobin to glutathionylhemoglobin has been proposed as another marker of oxidative stress [160].

Low-density lipoprotein (LDL) from uremic patients is more susceptible to oxidation than that from control subjects [161] (oxidized LDL – oxLDL). This chemically modified LDL is more readily accumulated in macrophages, which results in the development of foam cells, an early event in atherogenesis. LDL autoantibodies against oxLDL have been demonstrated in ESRD, especially in hemodialysed patients [162]. Oxidative modification of the protein moiety of LDL is a trigger of macrophage respiratory burst [163, 164].

Malondialdehyde levels are increased in ESRD [165]. The capacity of malondialdehyde to form DNA adducts [166] may play a patho-physiological role in carcinogenesis. Low dose IV folinic acid given to dialysis patients reduces the levels of serum malondialdehyde and thus improves the cardiovascular risk profile [167].

Several small molecular compounds might also be modified by oxidation. Organic chloramines are generated by the chemical binding of hypochlorite, a free radical produced by activated leukocytes, to retained organic compounds [168].

9) Peptides

Peptides constitute a heterogeneous group of molecules. In general, peptides can be considered as typical MM. $ß_2$-M and PTH have been discussed previously.

Granulocyte-inhibiting protein I (GIP I – 28 kD), recovered from uremic sera or ultrafiltrate, suppresses the killing of invading bacteria by polymorphonuclear cells [169]. The compound has structural analogy with the variable part of kappa light chains. Another peptide with a granulocyte-inhibitory effect (GIP II – 9.5 kD) is partially homologous with $ß_2$-M, and inhibits granulocyte glucose uptake and respiratory burst activity [170]. A degranulation-inhibiting protein (DIP – 24 kD), identical to angiogenin, was isolated from plasma ultrafiltrate of uremic patients [171]. The structure responsible for the inhibition of degranulation is different from the sites that are responsible for the angiogenic or ribonucleic activity of angiogenin. A structural variant of ubiquitin inhibits polymorphonuclear chemotaxis (chemotaxis-inhibiting protein – CIP – 8.5 kD) [172].

Atrial natriuretic peptide (ANP – 3.1 kD) and endothelin (3.5 kD) are elevated in dialysis patients, and may play a role in the regulation of the blood pressure [173]. ANP levels correlate with left atrial size, fluid overload, and decreased systemic clearance [174]. Endothelin causes peripheral insulin resistance, even at concentrations that induce no blood flow changes [175], and may play a role in uremic hypertension [176].

The opioid peptides ß-endorphin (3.5 kD), methionine-enkephalin (0.6 kD) and ß-lipotropin (1.9 kD) are elevated in dialysed patients [177]. Delta sleep-inducing peptide (0.9 kD) may modulate sleep-wakefulness [178].

Neuropeptide Y (NPY – 4.3 kD) is increased in uremia [179], and tends to increase further during hemodialysis [180]. It is a 36-amino acid peptide with renal vasoconstrictive activity [181]. Recently, plasma NPY was found to predict incident cardiovascular complications in end-stage renal disease [182]. NPY also acts as an orexigen [183]. Uremic patients with anorexia have lower NPY levels [183, 184]. The concentration of the anorexigen cholecystokinin (CCK) is increased in most patients with chronic renal failure [183].

Adrenomedullin, a 52-amino acid and potent hypotensive peptide, is found at markedly increased concentrations in chronic renal failure patients [185], and activates inducible nitric oxide synthase [186].

Cystatin C (13.3 kD), Clara cell protein (CC16) (15.8 kD) and the retinol-binding protein (RBP) (21.2 kD) are elevated in renal failure [187]. Cystatin C is an inhibitor of proteinases and cathepsins [188]. CC16 is an immunosuppressive α-microprotein [189].

Leptin, a 16 kD plasma protein decreases the appetite of uremic patients [190]. The rise in serum leptin is mostly attributed to decreased renal elimination [191], and is almost entirely limited to the free fraction [191]. Increased leptin is associated with low protein intake and loss of lean tissue in chronic renal failure [190]. Recent data suggest an inverted correlation between leptin and nutritional status [192], and a direct correlation with C-reactive protein (CRP) [193]. In CAPD-patients, serum leptin showed a progressive rise only in patients with body-weight loss [194]. Erythropoietin treatment results in a decline of leptinemia and an improvement of nutritional status [195].

However, leptin levels are also elevated in obese people and are hence not necessarily related to reduced appetite. Body fat and serum leptin also correlate in uremia [193]. Female gender and obesity are important factors that affect serum leptin also in ESRD-patients [196]. Don *et al.* suggest that in ESRD-patients leptin may be depressed during inflammation, and may actually act as a negative acute phase reactant [197]. Therefore, the biochemical role of leptin in renal failure remains inadequately defined.

Ghrelin is a recently described polypeptide hormone produced mainly by the stomach but also synthesized in various tissues including the kidney [198]. Ghrelin has been shown to stimulate a variety of nutrition-related effects, such as Growth Hormone (GH) release from the pituitary gland [199], increase in food intake [200], fat accumulation, and body weight gain [201]. A recent study described that plasma ghrelin was significantly increased in CKD patients compared with those in patients with normal renal function, and that plasma ghrelin was significantly correlated with both serum creatinine and GH. Mo-

reover, hemi-nephrectomy in mice caused a marked increase in the plasma ghrelin without significant changes in ghrelin mRNA levels in the stomach, suggesting that the kidneys are important in ghrelin clearance [198].

The status of ghrelin as uremic toxin can be doubted. Recent data point to the favourable effects of subcutaneously administered ghrelin on the nutriational condition of malnourished patients on peritoneal dialysis [202] and on the vascular status of rats in vivo and in vitro [203].

10) Phenols

Phenol depresses various functional parameters of enzymatic activity in polymorphonuclear leukocytes [204]. A depressive effect was demonstrated on the 3':5'-cyclic monophosphate response of the neostriatum to dopamine [205]. This effect was abolished after conjugation of phenol to phenylglucuronide. These findings may be relevant to hepatic and uremic coma. Phenol prevents in vitro the inhibition of parathyroid cell proliferation induced by calcitriol [206].

Phenols are lipophilic and protein-bound, and their removal by hemodialysis is markedly less than that of urea and creatinine [26]. Daily hemodialysis results in lower pre-dialysis serum levels compared to conventional alternate day dialysis [207]. In a hemodialysis setting, the removal of p-cresol and that of urea and creatinine are not correlated [26], demonstrating that the latter markers are not representative of the intra-dialytic behaviour of protein-bound compounds. Levels are markedly lower in PD, compared to hemodialysis [208].

Hypoalbuminemia and a rise in total concentration are correlated to an increase of the free active fraction [30]. A correlation between free p-cresol and hospitalization rate was demonstrated [30]. Patients hospitalized for infection also had a higher free p-cresol [30]. P-cresol also correlates with clinical uremic symptoms [209].

11) Phenylacetic acid

Phenylacetic acid (PAA) is a degradation product of the amino acid, phenylalanine. Plasma concentrations of PAA in patients with end-stage renal failure strongly exceed those in healthy controls. PAA was shown to inhibit iNOS expression and, consequently, NO production [210] and was identified as an inhibitor of Ca^{2+} ATPase activity in end-stage renal failure [211].

12) Purines

Uric acid, xanthine and hypoxanthine are the most important purines retained in uremia. The purines disturb calcitriol production and metabolization [212]. Administration of purines to animals results in a net decrease of serum calcitriol [212] and a decrease of uric acid in response to allopurinol administration results in a rise of plasma calcitriol levels [213]. Purines are involved in the resistance to calcitriol of immune competent cells [214]. Xanthine and

hypoxanthine have been implicated as modulators of neurotransmission, poor appetite and weight loss [215]. Both xanthine and hypoxanthine induce vasoconstriction [216] and disturb endothelial barriers [217].

Uric acid is a small water-soluble compound that is removed by hemodialysis from the plasma in a similar way as urea [218], but removal from the intracellular compartment is not nearly as efficient [219]. Dialytic removal of xanthine and hypoxanthine shows no correlation with that of urea and creatinine [218].

Conclusions

The uremic syndrome is the result of a complex set of biochemical and patho-physiological disturbances, emanating in a state of generalized malaise and dysfunction. This condition is related to the retention of a host of compounds; many of them exert a negative impact on key functions of the body; those molecules have consequently been identified as uremic toxins. Up to now, the toxic action of single solutes has repeatedly been studied, but the intermutual interference between compounds has rarely been considered. Although solute retention is one of the major patho-physiological events, deficiencies are functionally important as well.

Removal and generation of many compounds with proven biological or biochemical impact, especially toxins which are hydrophobic and/or not generated from protein break-down, can hardly be predicted by the intra-dialytic behaviour of urea, a current marker but a small water soluble compound generated from protein, with relatively little biological impact.

Solute clearance eventually reaches a plateau as dialyzer blood flow and/or dialysate flow are increased; this plateau is reached much sooner for molecules with a higher molecular weight. As a result, clearance of MM *stricto sensu* is relatively blood and dialysate flow independent. Only an increase of dialysis time, dialyzer surface area, ultrafiltration rates and/or dialyzer pore size can enhance their removal.

Removal of solutes that behave like larger molecules, due to their protein binding, multicompartmental distribution and/or lipophilicity, will be less affected by the use of high flux dialyzers and/or dialyzers with a larger pore size. To improve the clearances of these "new definition MM", it may be necessary to develop renal replacement systems with different characteristics, e.g. specific adsorption systems and/or procedures that allow a slower exchange of solutes.

Earlier concepts of adsorption, eventually largely abandoned, should perhaps be reconsidered, especially for the removal of organic acids. More specific and/or more efficient adsorptive systems may, however, be needed. As an

alternative, adsorption of toxins or of their precursors may be pursued at the intestinal level.

The next step is to pursue more specific removal. However, before this can be realised, we will need to know more about the toxic compounds responsible for these disturbances.

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Резиме

УРЕМИЧНИ ТОКСИНИ КАЈ ХРОНИЧНАТА БУБРЕЖНА ИНСУФИЦИЕНЦИЈА

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Уремичниот синдром е комплексна мешавина од органски дисфункции, кои се припишани на ретенцијата на безброј состојки кои, во нормални услови, се екскретирани преку здравите бубрези. Во текот на последните години направени се големи чекори во областа на идентификацијата и карактеризацијата на ретенционите уремични соединенија и во познавањето на нивната патофизиолошка важност, иако, спознанието останува далеку од комплетно. Во тековниот труд ќе биде издискутирана општата класификација базирана на нивната молекуларна тежина и нивните протеин-врзувачки карактеристики, со осврт на нивното отстра-

нување. Понатаму, ќе се направи преглед на тековното познавање на најважните уремични продукти и нивните клинички и биолошки ефекти.

Клучни зборови: уремични токсини, хронична бубрежна инсуфициенција, дијализа.

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Table 1: The uremic syndrome – main clinical alterations
Табела 1: Уремичен синдром - главни клинички алтерации

Cardiovascular system
atheromatosis
arteriosclerosis
cardiomyopathy
decreased diastolic compliance
hyper/hypotension
pericarditis
Nervous system
concentration disturbances
cramps
dementia
depression
fatigue
headache
motor weakness
polyneuritis
reduced sociability
restless legs
sleep disorders
stupor,coma
Hematological system / coagulation
anemia
bleeding
hypercoagulability
Immunological system
inadequate antibody formation
stimulataion of inflammation (baseline)
susceptibility to cancer
susceptibility to infection
Endocrinology
dyslipidemia
glucose intolerance
growth retardation
hyperparathyroidism
hypogonadism
impotence, diminished libido
Bone disease
adynamic bone disease
amyloidosis ($ß_2$-microglobulin)

defective calcitriol metabolism
osteitis fibrosa
osteomalacia
osteoporosis
Skin
melanosis
pruritus
uremic frost
Gastro-intestinal system
anorexia
dyspepsia
gastro-intestinal ulcers
hiccup
nausea, vomiting
pancreatitis
Pulmonary system
pleuritis
pulmonary edema
sleep apnoea syndrome
Miscellaneous
hypothermia
thirst
uremic foetor
weight loss

Table 2: Major uremic retention solutes and their molecular weight (Daltons)
Табела 2: Најважни ретенциони уремични соединенија и нивните молекуларни тежини (Далтони)

Compound	MW	Compound	MW
ADMA/SDMA	202	Adrenomedullin	5729
ANF	3080	Benzylalcohol	108
ß-endorphin	3465	ß-guanidinopropionic acid	131
ß2-microglobulin	11818	CGRP	3789
Cholecystokinin	3866	CIP	8500
Clara cell protein	15800	CML	188
CMPF	240	Complement factor D	23750
Creatine	131	Creatinine	113
Cystatin C	13300	Cytidine	234
DIP I	14400	DIP II	24000
3-deoxyglucosone	162	Dimethylarginine	202
Endothelin	4283	γ-guanidinobutyric acid	145
Glomerulopressin	500	GIP I	28000
GIP II	25000	Guanidine	59
Guanidinoacetic acid	117	Guanidinosuccinic acid	175
Hippuric acid	179	Homoarginine	188
Homocysteine	135	Hyaluronic acid	25000
Hypoxanthine	136	Imidazolone	203
Indole-3-acetic acid	175	Indoxyl sulfate	251
Leptin	16000	Melatonin	126
Methylguanidine	73	Myoinositol	180
Neuropeptide Y	4272	Orotic acid	156
Orotidine	288	o-OH-hippuric acid	195
Oxalate	90	p-cresol	108
p-OH-hippuric acid	195	Parathyroid hormone	9225
Pentosidine	135	Phenylacetylglutamine	264
Phenol	94	Phosphate	96
Pseudouridine	244	Putrescine	88
Retinol binding protein	21200	Spermine	202
Spermidine	145	Thymine	126
Trichloromethane	119	Tryptophan	202
Urea	60	Uric acid	168
Uridine	244	Xanthine	152

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