

Review on uremic toxins: Classification, concentration, and interindividual variability

RAYMOND VANHOLDER, RITA DE SMET, GRIET GLORIEUX, ANGEL ARGILÉS, ULRICH BAURMEISTER, PHILIPPE BRUNET, WILLIAM CLARK, GERALD COHEN, PETER PAUL DE DEYN, REINHOLD DEPPISCH, BEATRICE DESCAMPS-LATSCHA, THOMAS HENLE, ACHIM JÖRRES, HORST DIETER LEMKE, ZIAD A. MASSY, JUTTA PASSLICK-DEETJEN, MARIANO RODRIGUEZ, BERND STEGMAYR, PETER STENVINKEL, CIRO TETTA, CHRISTOPH WANNER, and WALTER ZIDEK, For the EUROPEAN UREMIC TOXIN WORK GROUP (EUTOX)

The Nephrology Section, Department of Internal Medicine, University Hospital, Ghent, Belgium; Institute of Human Genetics, Montpellier, France; MAT Adsorption Technologies, Obernburg, Germany; Nephrology – Internal Medicine, Ste. Marguerite Hospital, Marseille, France; Baxter Healthcare Corporation, Lessines, Belgium; Division of Nephrology, Department of Medicine, University of Vienna, Vienna, Austria; Department of Neurology, Middelheim Hospital, Laboratory of Neurochemistry and Behaviour, University of Antwerp, Antwerp, Belgium; Gambro Corporate Research, Hechingen, Germany; INSERM Unit 507, Necker Hospital, Paris, France; Institute of Food Chemistry, Technical University, Dresden, Germany; Nephrology and Medical Intensive Care, UK Charité, Campus Virchow-Klinikum, Medical Faculty of Humboldt-University, Berlin, Germany; Membrana GmbH, Obernburg, Germany; Division of Nephrology, CH-Beauvais, and INSERM Unit 507, Necker Hospital, Paris, France; Fresenius Medical Care, Bad Homburg, Germany; University Hospital Reina Sofia, Research Institute, Cordoba, Spain; Norrlands University Hospital, Medical Clinic, Umea, Sweden; Nephrology Department, University Hospital, Huddinge, Sweden; Division of Nephrology, University Hospital, Würzburg, Germany; and University Hospital Benjamin Franklin, Berlin, Germany

Review on uremic toxins: Classification, concentration, and interindividual variability.

Background. The choice of the correct concentration of potential uremic toxins for in vitro, ex vivo, and in vivo experiments remains a major area of concern; errors at this level might result in incorrect decisions regarding therapeutic correction of uremia and related clinical complications.

Methods. An encyclopedic list of uremic retention solutes was composed, containing their mean normal concentration (C_N), their highest mean/median uremic concentration (C_U), their highest concentration ever reported in uremia (C_{MAX}), and their molecular weight. A literature search of 857 publications on uremic toxicity resulted in the selection of data reported in 55 publications on 90 compounds, published between 1968 and 2002.

Results. For all compounds, C_U and/or C_{MAX} exceeded C_N . Molecular weight was lower than 500 D for 68 compounds; of the remaining 22 middle molecules, 12 exceeded 12,000 D. C_U ranged from 32.0 ng/L (methionine-enkephalin) up to 2.3 g/L (urea). C_U in the ng/L range was found especially for the middle molecules (10/22; 45.5%), compared with 2/68 (2.9%) for a molecular weight <500 D ($P < 0.002$). Twenty-five solutes

(27.8%) were protein bound. Most of them had a molecular weight <500 D except for leptin and retinol-binding protein. The ratio C_U/C_N , an index of the concentration range over which toxicity is exerted, exceeded 15 in the case of 20 compounds. The highest values were registered for several guanidines, protein-bound compounds, and middle molecules, to a large extent compounds with known toxicity. A ratio of $C_{MAX}/C_U < 4$, pointing to a Gaussian distribution, was found for the majority of the compounds (74/90; 82%). For some compounds, however, this ratio largely exceeded 4 [e.g., for leptin (6.81) or indole-3-acetic acid (10.37)], pointing to other influencing factors than renal function, such as gender, genetic predisposition, proteolytic breakdown, posttranslational modification, general condition, or nutritional status.

Conclusion. Concentrations of retention solutes in uremia vary over a broad range, from nanograms per liter to grams per liter. Low concentrations are found especially for the middle molecules. A substantial number of molecules are protein bound and/or middle molecules, and many of these exert toxicity and are characterized by a high range of toxic over normal concentration (C_U/C_N ratio). Hence, uremic retention is a complex problem that concerns many more solutes than the current markers of urea and creatinine alone. This list provides a basis for systematic analytic approaches to map the relative importance of the enlisted families of toxins.

Key words: uremic toxins, uremic toxicity, renal failure, concentrations, retention.

Received for publication July 3, 2002

and in revised form October 24, 2002

Accepted for publication December 13, 2002

© 2003 by the International Society of Nephrology

The uremic syndrome is attributed to the progressive retention of a large number of compounds, which under normal conditions are excreted by the healthy kidneys

[1–3]. These compounds are called uremic retention solutes, or uremic toxins, when they interact negatively with biologic functions.

A substantial number of publications have been devoted to the concentration changes of individual uremic retention solutes in several conditions of end-stage renal disease (ESRD). To our knowledge, no attempts have been made, however, to develop an in depth and systematic overview of all these data with the intention to open the field for new analytic approaches.

Various aspects of the uremic syndrome remain partly or entirely unexplored, although an exact knowledge of its nature, as well as of the relative importance of the various responsible toxins, is urgently needed to allow the development of better defined and more specific removal strategies than the empiric ones based on diffusion, convection, or molecular size exclusion, as applied in the current dialysis membranes today. The clinical importance to address this issue in depth has recently been stressed by the demonstration of an improved outcome when clearance of uremic toxins with a molecular weight range of approximately 1000 to 5000 D was enhanced [4].

In many studies, concentrations are applied that exceed those encountered in uremia, so that conclusions might have relatively little clinical relevance. To overcome these pitfalls, in the present publication, three lists are presented containing in total 90 retention solutes together with their normal and uremic concentrations, offering a “dictionary” of uremic retention solutes, in a standardized and homogeneous manner. The work has been constructed as an objective report of the available information, rather than as a critical analysis of how this information has been obtained. The report is followed by a number of reflections regarding the characteristics and the retention pattern of the depicted molecules. The aim is to offer a platform for more systematic future studies in the area of uremic toxicity.

METHODS

A database of 857 publications, published between 1966 and 2002, covering the field of uremic toxins and/or uremic toxicity, was considered. The references were collected based on a Medline literature search (Reference Manager 9) with as reference words (search items) “uremic toxins/uremic toxicity” and/or specific names of known retention solutes as recently reviewed [1, 3, 5]. This list was further completed based on the personal expertise of the authors. Among these publications, 149 were devoted to solute concentration. Only plasma/serum concentrations were taken into consideration.

A search was made to register a representative mean or median normal concentration, a representative mean or median uremic concentration, and the highest reported

single uremic concentration. Subsequently, 55 publications were used for the present analysis.

Compounds were included only if their mean/median uremic concentration and/or maximum uremic concentration were in excess of the reported normal concentration.

For the sake of uniformity, all concentration (C) values were normalized to a volume of 1 liter (L), and were reported in grams per liter (g/L), or, if less concentrated, in milligrams per liter (mg/L), micrograms per liter (μ g/L), or nanograms per liter (ng/L). Data that were reported in mole per liter (mol/L) or equivalent units were transformed to g/L or equivalent concentration values by the formula:

$$C \text{ (g/L)} = [C \text{ (mol/L)} \times \text{molecular weight}] \quad (\text{Eq. 1})$$

Units of concentration were chosen in a way that the uremic concentration always exceeded 1.0. All data were reported up to one digit below unity, unless if normal values or their standard deviations (SD) were smaller than 0.1. The molecular weights utilized might be slightly different from other reported values, depending on the methods and procedures applied to identify the molecular size [i.e., sodium dodecyl sulfate (SDS)-gel electrophoresis, gel permeation chromatography, mass spectroscopy, or others].

Uremic concentrations were all expressed as mean $\pm$ SD, or if means were lacking, as median. If literature data were expressed in mean $\pm$ SEM, SEM was transformed to SD according to the formula:

$$SD = SEM \times \sqrt{(n - 1)} \quad (\text{Eq. 2})$$

Our primary research was aimed at finding the highest reported mean/median uremic concentrations (C_U). These values were noted, regardless of whether the patients reported were in the predialytic stage, or dialysed by hemodialysis or peritoneal dialysis. If possible, the corresponding normal values (C_N) were extracted from the same publications. If not available in these specific publications, normal values were collected from other sources. If normal values were indicated in the publications as being below a given detection limit, this limit was introduced as the highest normal value. If normal values were given as a range, also the highest value was taken as the reference. In both cases, the normal value is accompanied by the symbol “<” in the tables. In any other case, means $\pm$ SD are given and the means were taken as the reference value. Per C_N , C_U , or C_{MAX} value, only one literature reference was finally to be used for data collection, so that per molecule maximum three references can be found. It, however, occurred only once that we had to refer to three publications (homocysteine), whereas the characteristic concentrations of most molecules were only covered by one reference ($N = 67$). For 22 molecules, two references were used.

In addition, if available, the single highest uremic concentration ever reported was illustrated as well (C_{MAX}).

This information was not necessarily obtained from the same publication as the mean/median uremic value. If no range or individual values could be found, a hypothetical maximal value was calculated as:

$$C_{\text{MAX}} = \text{Mean} + 2 \text{ SD} \quad (\text{Eq. 3})$$

Finally, the number of patients on which the C_N and C_U values had been obtained, the molecular weight, the reference(s) from which the data had been collected, and (if available) the larger group to which the molecules belonged (e.g., guanidines, phenols, peptides) were reported.

Molecules were subdivided into three major classes: (1) small solutes (<500 D) with no known protein binding; (2) solutes with known or likely protein binding; and (3) middle molecules (≥ 500 D). For all the protein-bound solutes, only the total concentrations were illustrated.

The lower cutoff value for the so-called middle molecules (500 D) was based on the original literature data reporting on this class of uremic solutes [6]. In this literature, middle molecule molecular weight range is usually reported as 500 to 2000 D. Since most of the identified larger uremic solutes are characterized by a molecular weight in excess of 2000 D, the upper limit for middle molecules was not restricted to 2000 D, but all solutes with a molecular weight in excess of 2000 D were also enlisted as middle molecules.

Molecules that are not filterable through the glomerular basement membrane because of their molecular size (molecular weight $> \sim 60,000$ D) were not included [e.g., lipoprotein(a)]. Likewise, anorganic compounds were excluded, although it is acknowledged that they can exert toxicity (e.g., H_2O , K^+ , phosphate, trace elements). The reader is referred for these compounds to specific publications [7–9].

Furthermore, the ratio between mean uremic and normal concentration was calculated (C_U/C_N) to obtain an index of the relative increase during uremia. In addition, also the ratio of maximal over mean uremic concentration (C_{MAX}/C_U) was calculated. The latter index describes whether data distribution was Gaussian or not.

For some of the advanced glycation end products (AGEs), published concentrations are expressed in the literature as picogram per milligram (pg/mg) protein or microgram per milligram ($\mu\text{g}/\text{mg}$) protein [10, 11]. Since in these publications, however, no serum total protein was mentioned, it was impossible to extrapolate the absolute AGE concentrations. In these cases, absolute content was calculated by multiplying with an average serum protein concentration (i.e., 70,000 mg/L). The reported AGE values all were obtained in uremic patient groups without diabetes mellitus.

For statistical comparison, an Instat® statistical package was used. Sets of values reported per group of molecules (small water-soluble, protein-bound, middle mole-

cules) were compared statistically to each other by the Mann-Whitney U test. Dichotomous variables were compared by chi-square test. Statistical significance was accepted for $P < 0.05$.

RESULTS

The concentrations of in total 90 uremic solutes are listed in Tables 1 to 3. In Table 1, results regarding 45 low molecular weight solutes (molecular weight <500 D) without known protein binding (small free water-soluble compounds) are summarized [12–33]. Similarly, Table 2 contains data on 25 compounds with known protein binding or belonging to groups of solutes that are known to be protein bound [10, 11, 20, 34–53]. Most of these protein-bound solutes are also characterized by a molecular weight <500 D, although two of these compounds (leptin and retinol-binding protein) have a molecular weight conforming with that of the middle molecules. For these protein-bound compounds, the tables only contain total (free + bound) concentrations. Table 3 then contains information on the middle molecules ($N = 22$) [22, 45, 53–66].

Hence, 68 solutes are characterized by a molecular weight below 500 D. Among the 22 middle molecules, 12 (54.5%) have a molecular weight $> 12,000$ D.

For several molecules, we found a discordance between the highest [13, 34, 63] and the lowest range of reported uremic concentrations [15, 36, 67]. The most striking examples [asymmetric dimethylarginine (ADMA); interleukin-6 (IL-6); 3-deoxyglucosone (3-DG)] are illustrated in Table 4, where, in each instance, a discrepancy by a factor of 8 or more was observed. In Tables 1 to 3 only the highest reported value is illustrated. It is of note that for ADMA, values intermediate between the lower range as observed by Marescau et al [15] and the higher range observed by Kang et al [13] have been reported by Vallance et al [14] (approximately 878.5 $\mu\text{g}/\text{L}$) and Zoccali et al [68] (777.7 $\mu\text{g}/\text{L}$).

The uremic concentration of the 90 reported molecules was spread over a broad range, from 2.3 g/L [urea, full urea concentration; for blood urea nitrogen (BUN) multiply by 28/60] to 32.2 ng/L (methionine-enkephalin) (factor $> 70 \times 10^4$). The concentration range for the small water-soluble compounds ($N = 45$) extended from 2.3 g/L (urea) to 62.7 ng/L (β -lipotropin) (Table 1). The range for the protein-bound solutes ($N = 25$) extended from 247.0 mg/L (hippuric acid) to 175.8 ng/L (melatonin) (Table 2), and for the middle molecules ($N = 22$) from 192.0 mg/L (retinal-binding protein) to 32.2 ng/L (methionine-enkephalin) (Table 3). The median concentration value was 773.8 $\mu\text{g}/\text{L}$ overall (methylguanidine) and was 1.2 mg/L for the small water-soluble compounds, as well as for the protein-bound compounds, but only 0.95 $\mu\text{g}/\text{L}$ for the middle molecules. Considering these medians, in general, the concentration of the middle molecules was

Table 1. Free water-soluble low-molecular-weight solutes ($N = 45$)

Solute	C _N	C _U	C _{MAX}	MW	Ref	Group
1-methyladenosine $\mu\text{g/L}$	17.1 $\pm$ 5.1/10	104.0 $\pm$ 56.2/17	216.4	281	[12]	Ribonucleosides
1-methylguanosine $\mu\text{g/L}$	13.7 $\pm$ 16.9/10	41.6 $\pm$ 23.8/17	89.2	297	[12]	Ribonucleosides
1-methylinosine $\mu\text{g/L}$	13.5 $\pm$ 3.9/10	620.4 $\pm$ 203.4/14	1027.2	282	[12]	Ribonucleosides
ADMA mg/L	0.2 $\pm$ 0.06/6	1.6 $\pm$ 1.2/10	7.3 ^a	202	[13, 14]	Guanidines
α -keto- δ -guanidinovaleric acid $\mu\text{g/L}$	<30.2/66	—	140.4 ^a	151	[15]	Guanidines
α -N-acetylarginine $\mu\text{g/L}$	18.1 $\pm$ 24.8/16	328.3 $\pm$ 142.6/13	4580.0 ^a	216	[16, 17]	Guanidines
Arab(in)itol mg/L	<0.6/33	15.0 $\pm$ 9.0/12	33.0	152	[18, 19]	Polyols
Argininic acid $\mu\text{g/L}$	<77.0/66	80.5 $\pm$ 56.0/11	197.8 ^a	175	[15, 16]	Guanidines
Benzylalcohol mg/L	—	27.0 $\pm$ 50.7/17	187.9 ^a	108	[20]	
β -guanidinopropionic acid $\mu\text{g/L}$	<3.3/24	28.8 $\pm$ 18.3/29	65.4	131	[21]	Guanidines
β -lipotropin ng/L	<55.3/10	62.7/22	108.8 ^a	461	[22]	Peptides
Creatine mg/L	9.7 $\pm$ 3.3/24	134.0 $\pm$ 30.3/29	235.8 ^a	131	[21]	Guanidines
Creatinine mg/L	<12.0/23	136.0 $\pm$ 46.0/19746	240.0 ^a	113	[23, 24]	Guanidines
Cytidine $\mu\text{g/L}$	<468.0	683.3 $\pm$ 287.8/7	1263.6 ^a	234	[25]	Purines
Dimethylglycine $\mu\text{g/L}$	<381.1/33	576.8/18	1040.3 ^a	103	[26]	
Erythritol mg/L	<0.7/33	9.8 $\pm$ 14.0/12	37.0 ^a	122	[18, 19]	Polyols
γ -guanidinobutyric acid $\mu\text{g/L}$	<3.6/24	33.3 $\pm$ 16.0/30	1750.0 ^a	145	[27, 17]	Guanidines
Guanidine $\mu\text{g/L}$	<11.8/16	172.9 $\pm$ 83.8/13	800.0 ^a	59	[16, 17]	Guanidines
Guanidinoacetic acid $\mu\text{g/L}$	222.3 $\pm$ 79.6/24	383.8 $\pm$ 143.9/29	693.8 ^a	117	[21]	Guanidines
Guanidinosuccinic acid mg/L	0.03 $\pm$ 0.01/16	6.5 $\pm$ 3.4/13	47.0 ^a	175	[16, 17]	Guanidines
Hypoxanthine mg/L	1.5 $\pm$ 0.5/145	2.0 $\pm$ 1.6/65	5.3	136	[28, 29]	Purines
Malondialdehyde $\mu\text{g/L}$	257.7 $\pm$ 81.7/30	428.8 $\pm$ 170.4/16	769.6	71	[30]	
Mannitol mg/L	<1.3/33	26.0 $\pm$ 25.0/12	76.0	182	[18, 19]	Polyols
Methylguanidine $\mu\text{g/L}$	<7.3/24	773.8 $\pm$ 508.8/5	1820.0 ^a	73	[21, 17]	Guanidines
Myoinositol mg/L	<10.0/8	94.0 $\pm$ 69.0/12	232.0	180	[18]	Polyols
N ² ,N ² -dimethylguanosine $\mu\text{g/L}$	9.0 $\pm$ 4.7/10	236.4 $\pm$ 89.7/14	415.8	311	[12]	Ribonucleosides
N ⁴ -acetylcytidine $\mu\text{g/L}$	57.0 $\pm$ 17.1/10	159.6 $\pm$ 30.8/14	221.2	285	[12]	Ribonucleosides
N ⁶ -methyladenosine $\mu\text{g/L}$	18.5 $\pm$ 8.4/10	70.3 $\pm$ 53.3/17	176.9	281	[12]	Ribonucleosides
N ⁶ -threonylcarbamoyladenine $\mu\text{g/L}$	35.5 $\pm$ 27.2/10	378.0 $\pm$ 151.2/17	680.4	378	[12]	Ribonucleosides
Orotic acid mg/L	0.5 $\pm$ 1.4/30	6.7 $\pm$ 16.0/22	38.7	174	[31]	Pyrimidines
Orotidine mg/L	1.2 $\pm$ 1.6/30	20.2 $\pm$ 13.5/22	47.2	288	[31]	Pyrimidines
Oxalate mg/L	0.3 $\pm$ 0.1/8	4.9 $\pm$ 1.4/8	7.6	90	[32]	
Phenylacetylglutamine mg/L	<4.7	53.3 $\pm$ 44.7/6	120.6 ^a	264	[33]	
Pseudouridine mg/L	0.5 $\pm$ 5.8/30	13.1 $\pm$ 21.4/7	86.6 ^a	244	[25, 31]	Ribonucleosides
SDMA $\mu\text{g/L}$	76.1 $\pm$ 21.0/66	640.3 $\pm$ 212.1/38	1232.2 ^a	202	[15]	Guanidines
Sorbitol mg/L	<0.4/33	3.1 $\pm$ 2.1/12	7.3	182	[18, 19]	Polyols
Taurocyamine $\mu\text{g/L}$	<52.2/24	—	121.8 ^a	174	[17]	Guanidines
Threitol $\mu\text{g/L}$	<319.6/33	990.0 $\pm$ 920.0/12	5697.4 ^a	122	[18, 19]	Polyols
Thymine mg/L	—	2.8 $\pm$ 4.2/22	11.2	126	[31]	Pyrimidines
Uracil $\mu\text{g/L}$	<224.0	252.0 $\pm$ 154.6/7	448.0 ^a	112	[25]	Purines
Urea g/L	<0.4/23	2.3 $\pm$ 1.1/16	4.6 ^a	60	[24]	
Uric acid mg/L	<67.2	83.4 $\pm$ 44.5/7	146.7 ^a	168	[25]	Purines
Uridine mg/L	1.5 $\pm$ 1.3/30	9.8 $\pm$ 11.4/22	32.6	244	[31]	Pyrimidines
Xanthine mg/L	0.5 $\pm$ 1.4/180	1.5 $\pm$ 0.8/65	3.0	152	[28, 29]	Purines
Xanthosine $\mu\text{g/L}$	23.9 $\pm$ 12.8/10	96.6 $\pm$ 62.9/11	222.4	284	[12]	Ribonucleosides

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; ADMA, asymmetrical dimethylarginine; SDMA, symmetrical dimethylarginine. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means $\pm$ SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means $\pm$ SD or, in the case of a single value, as a median.

^aC_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

lower compared to the water-soluble and protein-bound solutes and this was confirmed by comparative statistical analysis ($P = 0.01$ vs. small water-soluble compounds; $P = 0.02$ vs. protein-bound compounds). For 10 out of 22 of the middle molecules (45.5%) uremic concentration was in ng/L range, compared with 1 out of 45 small water-soluble compounds (2.2%; $P < 0.001$) and 1 out of 25 protein-bound compounds (2.5%; $P < 0.01$).

The index C_U/C_N offers an indication about the differences between uremic and normal concentration values, over which toxicity can be exerted. The 20 solutes scoring higher than 15 and the 19 solutes scoring lower than 2.5

are listed in Table 5. The cutoff values for these lists were set arbitrarily. Among the eight compounds with the highest C_U/C_N index, there were two guanidines, three protein-bound solutes, and two middle molecules. Among the 20 lowest scoring compounds, there were seven middle molecules. When considering the index per group of molecules, only the difference between protein-bound molecules and middle molecules was at the borderline of significance ($P = 0.06$).

The index C_{MAX}/C_U indicates which solutes show a non-Gaussian distribution, in a way that the highest ratios are obtained for the solutes with the most abnormal

Table 2. Protein-bound solutes ($N = 25$)

Solute	C _N	C _U	C _{MAX}	MW	Ref	Group
2-methoxyresorcinol $\mu\text{g/L}$	—	$19.6 \pm 81.2/17$	322.0^a	140	[20]	Phenols
3-deoxyglucosone mg/L	$0.3 \pm 0.1/30$	$1.7 \pm 1.0/27$	3.5	162	[34]	AGE
CMPF mg/L	$7.7 \pm 3.3/7$	$61.0 \pm 16.5/15$	94.0^a	240	[35]	
Fructoselysine mg/L	—	$58.1 \pm 10.8/10$	79.7	308	[10]	AGE
Glyoxal $\mu\text{g/L}$	67.0 ± 20.0	$221.0 \pm 28.0/20$	277.0	58	[36]	AGE
Hippuric acid mg/L	<5.0	$247.0 \pm 112.0/7$	471.0	179	[37]	Hippurates
Homocysteine mg/L	$<1.7/24$	$8.1 \pm 1.6/7$	26.4^a	135	[38–40]	
Hydroquinone $\mu\text{g/L}$	—	$50.6 \pm 84.7/17$	286.0^a	110	[20]	Phenols
Indole-3-acetic acid $\mu\text{g/L}$	$17.5 \pm 17.5/7$	$875.0 \pm 560.0/42$	9076.9^a	175	[41,42]	Indoles
Indoxyl sulfate mg/L	$0.6 \pm 5.4/40$	$53.0 \pm 91.5/20$	236.0	251	[35]	Indoles
Kinurenine $\mu\text{g/L}$	$<391/7$	$686.4 \pm 178.9/21$	952.6	208	[43]	Indoles
Kynurenine acid mg/L	<1.0	—	9.5^a	189	[44]	Indoles
Leptin $\mu\text{g/L}$	$8.4 \pm 6.7/56$	$72.0 \pm 60.6/8$	490.0^a	16000	[45, 46]	Peptides
Melatonin ng/L	$26.5 \pm 7.1/35$	$175.8 \pm 130.2/13$	436.2	126	[47]	Indoles
Methylglyoxal $\mu\text{g/L}$	$47.0 \pm 12.0/15$	$110.0 \pm 18.0/20$	146.0	72	[36]	AGE
N ^ε -(carboxymethyl)lysine mg/L	$1.1 \pm 0.3/24$	$4.3 \pm 1.3/44$	6.9	204	[11]	AGE
p-cresol mg/L	$0.6 \pm 1.0/12$	$20.1 \pm 10.3/20$	40.7	108	[48]	Phenols
Pentosidine $\mu\text{g/L}$	$51.6 \pm 18.8/19$	$896.0 \pm 448.0/24$	2964.0^a	342	[49]	AGE
Phenol mg/L	$0.6 \pm 0.2/12$	$2.7 \pm 3.9/10$	10.5	94	[48]	Phenols
P-OHhippuric acid mg/L	—	$18.3 \pm 6.6/13$	31.5	195	[50]	Hippurates
Putrescine $\mu\text{g/L}$	$21.1 \pm 7.9/10$	$77.4 \pm 27.3/25$	132.0	88	[51]	Polyamines
Quinolinic acid mg/L	$0.1 \pm 0.05/10$	$1.5 \pm 0.9/54$	3.3	167	[52]	Indoles
Retinol-binding protein mg/L	<80	$192.0 \pm 78.0/112$	369.2^a	21200	[53]	Peptides
Spermidine $\mu\text{g/L}$	—	$97.2 \pm 45.0/25$	187.2	145	[51]	Polyamines
Spermine $\mu\text{g/L}$	—	$18.2 \pm 16.2/25$	66.7^a	202	[51]	Polyamines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference; CMPF, 3-carboxy-4-methyl-5-propyl-2-furanpropionic acid; AGE, advanced glycation end products. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. Normal values are reported as means $\pm$ SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as means $\pm$ SD.

^aC_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U).

Table 3. Middle molecules ($N = 22$)

Solute	C _N	C _U	C _{MAX}	MW	Ref	Group
Adrenomedullin ng/L	$13.2 \pm 4.6/17$	$41.8 \pm 19.7/29$	81.2	5729	[54]	Peptides
Atrial natriuretic peptide ng/L	$28.0 \pm 12.2/23$	$202.0 \pm 117.3/27$	436.6	3080	[55]	Peptides
β_2 -microglobulin mg/L	<2.0	$55.0 \pm 7.9/10$	100.0^a	11818	[53, 56]	Peptides
β -endorphin ng/L	$<173.3/10$	$301.5/22$	492.0^a	3465	[22]	Peptides
Cholecystokinin ng/L	<20.0	$45.9 \pm 32.3/38$	131.5^a	3866	[57]	Peptides
Clara cell protein (CC16) mg/L	<0.1	$3.3 \pm 2.0/112$	12.5^a	15800	[53]	Peptides
Complement factor D mg/L	$1.9 \pm 0.5/5$	$19.8 \pm 4.1/5$	26.0^a	23750	[58]	
Cystatin C mg/L	<1.6	$11.8 \pm 3.0/112$	20.0^a	13300	[53]	Peptides
Degranulation inhibiting protein I ^c $\mu\text{g/L}$	$321.7 \pm 59.7/23$	$713.7 \pm 390.0/125$	1631.4^a	14100	[59] ^b	Peptides
Delta-sleep inducing peptide $\mu\text{g/L}$	—	$1.5 \pm 0.9/7$	3.3	848	[60]	Peptides
Endothelin ng/L	$20.8 \pm 3.8/23$	$63.0 \pm 33.2/12$	129.4	4283	[55]	Peptides
Hyaluronic acid $\mu\text{g/L}$	$<124.0/86$	$215.0 \pm 257.0/184$	1843.0^a	25000	[61]	Peptides
Interleukin-1 β ng/L	$<160.0/15$	$428.0 \pm 134.0/29$	1700.0	32000	[62]	Cytokines
Interleukin-6 ng/L	$13.3 \pm 3.1/28$	$92.3 \pm 117.9/230$	328.1	24500	[63]	Cytokines
κ -Ig light chain mg/L	$34.0 \pm 15.0/15$	$70.0 \pm 60.9/104$	287.0^a	25000	[64]	Peptides
λ -Ig light chain mg/L	$31.0 \pm 11.2/15$	$87.0 \pm 60.9/104$	328.0^a	25000	[64]	Peptides
Leptin $\mu\text{g/L}$	$8.4 \pm 6.7/56$	$72.0 \pm 60.6/8$	490.0^a	16000	[45, 46]	Peptides
Methionine-enkephalin ng/L	$<18.3/10$	$32.2/22$	75.5^a	555	[22]	Peptides
Neuropeptide Y ng/L	<80.0	$64.9 \pm 25.5/19$	115.9	4272	[57]	Peptides
Parathyroid hormone $\mu\text{g/L}$	<0.06	$1.2 \pm 0.6/10$	2.4	9225	[65]	Peptides
Retinol-binding protein mg/L	<80	$192.0 \pm 78.0/112$	369.2^a	21200	[53]	Peptides
Tumor necrosis factor- α ng/L	$13.3 \pm 3.0/28$	$114.0 \pm 147.0/230$	408.0	26000	[63, 66]	Cytokines

Abbreviations are: C_N, normal concentration; C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; MW, molecular weight; ref, reference. The underlined numbers behind the slash point to the number of data on which the means or medians have been obtained. No underlined number indicates that no data about the number of samples were available. No number indicates that no n value was given. Normal values are reported as mean $\pm$ SD, or in the case of a single value as a maximum (accompanied by <); uremic values are reported as mean $\pm$ SD or, in the case of a single value, as a median.

^aC_{MAX} values are original data (all other values were calculated as mean + 2 SD based on C_U)

^bS Schmaldienst, Vienna: personal communication

^cDegranulation inhibiting protein I corresponds to angiogenin

Table 4. Highest versus lowest reported concentrations

	Low range C _U	Ref	High range C _U	Ref	Low range C _{MAX}	Ref	High range C _{MAX}	Ref
ADMA $\mu\text{g/L}$	169.5 $\pm$ 31.2	[15]	1616.0 $\pm$ 1212.0	[13]	282.8	[15]	7272.0	[13]
IL-6 ng/L	11.7 $\pm$ 2.8	[67]	92.3 $\pm$ 117.9	[63]	80.0	[67]	328.1	[63]
3-DG $\mu\text{g/L}$	59.0 $\pm$ 13.0	[36]	1715.0 $\pm$ 1009.6	[34]	85.0	[36]	3500	[34]

Abbreviations are: C_U, mean/median uremic concentration; C_{MAX}, maximal uremic concentration; ADMA, asymmetrical dimethylarginine; IL-6, interleukin-6; 3-DG, 3-deoxyglucose.

Table 5. C_U/C_N ratio: Highest and lowest scoring molecules

20 solutes scoring ≥ 15		19 solutes scoring < 2.5	
Guanidinosuccinic acid	216.67	Retinol-binding protein	2.40 ^a
Methylguanidine	106.00 ^a	Methylglyoxal	2.34
Indoxyl sulfate	88.33	Cholecystokinin	2.30 ^a
Indole-3-acetic acid	50.00	3-deoxyglucose	2.27
Hippuric acid	49.40 ^a	Degranulation inhibiting protein I	2.21
1-methylinosine	45.96	κ -Ig light chain	2.20
<i>p</i> -cresol	33.50	Methionine-enkephalin	1.76 ^a
Clara cell protein (CC16)	33.00 ^a	Kinurenine	1.76 ^a
β_2 -microglobulin	27.50 ^a	ADMA	1.75
N ² ,N ² -dimethylguanosine	26.27	β -endorphin	1.74 ^a
Pseudouridine	26.20	Hyaluronic acid	1.73 ^a
Arab(in)itol	25.00 ^a	Guanidinoacetic acid	1.73
Parathyroid hormone	20.00 ^a	Dimethylglycine	1.51 ^a
Mannitol	20.00 ^a	Cytidine	1.46 ^a
Interleukin-6	19.50	Hypoxanthine	1.33
α -N-acetylgarginine	18.14	Uric acid	1.24 ^a
Pentosidine	17.36	β -Lipotropin	1.13 ^a
Orotidine	16.83	Argininic acid	1.05 ^a
Oxalate	16.33	Neuropeptide Y	0.81 ^a
Quinolinic acid	15.00		

Abbreviations are: C_U, mean/median uremic concentration; C_N, normal concentration; ADMA, asymmetrical dimethylarginine. The cut-off values of 15 and 2.5 were set arbitrarily. Solute with a high score display a high differential concentration, uremic vs. normal, over which biologic activity (toxicity) can be exerted. Conversely, this range is limited for solutes displaying a low value.

^aC_U/C_N obtained with C_N as a maximal (not as a mean) value

Table 6. C_{MAX}/C_U ratio: Highest scoring molecules

γ -Guanidinobutyric acid	52.55	Pseudouridine	6.61
2-Methoxyresorcinol	16.43	Oxalate	6.55
α -N-acetylgarginine	13.95	Orotic acid	5.78
Indole-3-acetic acid	10.37	Threitol	5.75
Hyaluronic acid	8.57	Hydroquinone	5.65
Guanidinosuccinic acid	7.23	Guanidine	4.63
Benzylalcohol	6.96	Indoxyl sulfate	4.45
Interleukin-6	6.84	Erythritol	4.11
Leptin	6.81	Thymine	4.00

Abbreviations are: C_{MAX}, maximal uremic concentration; C_U, mean/median uremic concentration.

distribution. In Table 6, the compounds with a C_{MAX}/C_U above or equal to 4.0 are displayed. It contains 17 compounds, four of which are guanidines, but also some middle molecules, such as leptin (6.81), IL-6 (6.84), and hyaluronic acid (8.57) are included. We found no statistically significant differences among groups.

Finally, in Table 7, a number of molecules to be considered for the future are listed. They do not figure in Tables 1 to 3 because either their uremic concentration is not

Table 7. Solute to be considered for the future

1-alkyl-2-formyl-3,4-glycosyl-pyrrole
2-(2-fuoryl)-4(5)-(2-furanyl)-1H-imidazole
3-deoxyfructosone
3-hydroxykinurenine
4-hydroxynonenal
Advanced oxidation protein products (AOPP)
Advanced glycation end products- β_2 -microglobulin
Anthranilic acid
β_2 -microglobulin fragments
Cadaverine
Crossline
Dimethylamine
Guanosine
Imidazolone
Malonaldehyde
Malondialdehyde
Methylamine
N ^c -carboxyethyllysine
Organic chloramines
Oxidized low-density-lipoprotein (oxLDL)
Parathyroid hormone fragments
Pyrraline
Pyrrole aldehyde
Trimethylamine

exactly known and/or because their uremic retention has not been proven.

DISCUSSION

This literature search essentially offers an overview of the currently known uremic retention solutes, together with their reported concentrations. Furthermore, a number of physicochemical characteristics and relative differences between uremic and normal concentrations and between maximal and mean uremic concentrations are considered as well.

The resulting lists are a striking illustration of the complexity of uremic retention. Obviously, there is more than the retention of a single compound or even a group of compounds, and also, there is more than the retention of the current markers, urea and creatinine. Without any doubt, clinical uremic toxicity is even more complex, since a host of retained compounds remain unidentified.

In the present inventory, uremic solutes are listed and classified according to the characteristics that potentially influence their removal pattern during dialysis or other methods of extracorporeal elimination (i.e., molecular weight and lipophilicity/hydrophilicity resulting in pro-

tein binding). The publication does not deal with biochemical or clinical relevance of the listed compounds, which has been presented by the same group of authors elsewhere [3]. The major practical impact of this work is as a tool for the future for any effort of in vitro or in vivo evaluation of uremic toxicity. Furthermore, the information collected here might also be of help for kinetic evaluation and modeling of uremic toxin time courses for generation rate and their appearance in different compartments.

Our data clarify the need to approach the problem of uremic toxicity in a different way than has been done in the past, as analyses were essentially restricted to the evaluation of one or a few molecules regarding their impact on one or a few biologic systems. The consideration of several compounds or groups of compounds at a time might conform more with reality. Hence, to solve the problem of uremic toxicity, collaborative studies involving different research groups with different scopes of interest will be needed. Another aspect to be considered for the future is the intermutual interference of uremic compounds, which might necessitate the study of solutes in a "uremic" rather than a "normal" milieu.

It is of note that molecules with a molecular weight exceeding 60,000 D (cutoff of the glomerular basement membrane) and anorganic substances were excluded from the analysis. This, for example, eliminated lipoprotein (a) (molecular weight $\sim 100,000$ D), despite an enhanced concentration in uremia [69, 70], and a potential for biologic activity. Likewise, phosphate was excluded, despite a potential link to cardiovascular morbidity/mortality [9, 71].

Essentially, we discerned small water-soluble compounds with a low molecular weight (< 500 D), small protein-bound solutes, and middle molecules (≥ 500 D). The two latter types of molecules are characterized by a reduced removal during standard dialysis with small pore, low-flux membranes [72, 73], compared to the small water-soluble compounds. Although not definitely conclusive, circumstantial evidence suggests that membranes with a larger pore size are not only linked to a more efficient removal of larger molecules [73–75], but also to an improvement of clinical status and/or survival [4, 76–78]. It is of note that a recent prospective study could not demonstrate, however, any difference in survival outcome in patients treated with large pore vs. small pore dialyzers [79].

Protein-bound molecules, on the other hand, are not very efficiently removed by any dialytic procedure [72]. As a consequence, their concentration can hardly be influenced in ESRD, so that their clinical impact could never be studied appropriately in dialyzed uremics.

AGEs were classified among the protein-bound solutes. AGEs have been shown to be bound to albumin and other proteins and may even change albumin struc-

ture and, hence, also its binding capacity for other ligands [80]. Nevertheless, the relation with the binding proteins is probably different from that of other classical protein-bound solutes, such as indoxyl sulfate or *p*-cresol. The binding should be considered as more long-lived than for the other ligands, which display a continuous and dynamic competition among each other and with drugs for the protein-binding sites [81–83]. In some publications, not only total concentrations but also free concentrations are reported for the AGEs [11, 56]. These values are by definition much lower than the total concentration. More than 250-fold lower values were found for furosine, the free fraction of fructoselysine ($201.1 \pm 73.1 \mu\text{g/L}$ vs. $58.1 \pm 10.8 \text{ mg/L}$) [11]. The tables only contain total (free + bound) concentrations.

One should be aware of the fact that the biologic action of protein-bound compounds is exerted by their free fraction. Hence, the total concentrations, as depicted in the present tables, are only valid if the experimental medium contains a sufficient quantity of albumin (or plasma protein). For in vitro experiments, it might be worthwhile to measure the free fraction in the final medium; however, usually only one solute will be added at a time, whereas in vivo many ligands will be present together, competing for the protein-binding sites and increasing the free fraction.

Overall, molecular weights of the listed compounds extended from 60 D (urea) to 32,000 D (IL-1 β). Also concentrations extended over a broad range with as highest value urea, in the g/L range, and as the lowest methionine-enkephalin, in the ng/L range. A high concentration is not necessarily related to a strong biologic activity. The two molecules with the highest concentrations (urea and creatinine) are known for their relatively limited biologic activity [1, 3]. Addition of urea to the dialysate at concentrations exceeding those encountered in uremia had relatively little impact on the clinical status of the patients submitted to this regimen [84].

The index C_U/C_N offers an indication on the differences between uremic and normal values. The larger the gap between the two values, and the larger the index, the larger the range over which biologic activity can be exerted, and the bigger the chance that biologic effects become apparent in vivo or in vitro. It is of note that a high C_U/C_N is to a large extent characteristic of guanidines, protein-bound solutes, and middle molecules (altogether 13/20 of the solutes scoring > 15), whereas many of these compounds exert substantial biologic activity [1, 3]. Furthermore, all these molecules are characterized by a different intradialytic behavior compared to the classical marker urea [27, 72, 73].

For protein-bound molecules the ratio C_U/C_N might even become more important for the unbound and biologically active fraction, than for total concentration, as has been observed for *p*-cresol [85].

Hence, removal strategies should be designed in a way that not only the standard molecules, but also other molecules that might be more important in the deterioration of the clinical condition, can be removed efficiently. A typical example is cardiovascular morbidity and mortality, at present one of the most worrying complications of the uremic condition [3]. Careful analysis of the literature shows that most molecules held as yet responsible for uremic toxicity are removed in a different way than urea and creatinine, either due to their molecular weight, protein binding, and/or multicompartmental behavior [1, 3]. Subsequently, dialytic removal strategies should be adapted accordingly, based on a global knowledge of the compounds playing a role in any complication of uremia.

The index C_{MAX}/C_U indicates which solutes follow a non-Gaussian distribution. The higher the value of this index, the more abnormal the distribution of concentrations. This might be the consequence of various interfering factors that are not necessarily related to the uremic status (e.g., gender, genetic predisposition, general condition, body composition, or nutritional status) [46, 86–88]. Several guanidines and middle molecules show a strong trend for abnormal distribution. Among these, IL-6 and leptin may have an important potential for biologic activity [3].

It is of note that for certain solutes a substantial gap exists between the highest reported mean concentrations [13, 34, 63] and the other reported concentrations [15, 36, 67]. This is the case for ADMA, IL-6, and 3-DG (Table 4). We considered it beyond the scope of this work to offer a critical analysis on the origin(s) of these differences in concentrations. Overestimation of true values is possible if tests are too sensitive [e.g., for certain types of enzyme-linked immunosorbent assay (ELISA)], if in chromatographic analysis [e.g., high-performance liquid chromatography (HPLC)] peak heights are overestimated because several compounds elute at the same moment or if external contamination has taken place. On the other hand, true values might be underestimated if extraction or derivatization is inefficient or if compound is lost during the preparative steps or analytic procedures (e.g., absorption on chromatographic columns or evaporation). Whether the discrepancies reported in Table 4 are attributable to technical reasons, or to differences in patient populations or removal strategies, is left open for discussion. If these concentrations are to be used for in vivo or vitro experiments, it is left to the reader which concentration to use, but he or she should be aware of these discrepancies and take them into account when interpreting the results.

Although we tried to make this database as complete as possible, it might be not exhaustive. Also in the future, new publications will appear on newly discovered compounds and/or known compounds will be presented with

different mean uremic or highest values than the ones reported here. For that matter, this database will need continuous updating (see **Acknowledgments**). Nevertheless, the present data offer, in our opinion, a broad overview of the present state of the art, and such an overview has, to our knowledge, never been published before. The authors will appreciate receiving additional information about new solutes to be added to the lists, or about enlisted molecules with reported concentrations that are different from the ones that are cited at present, enabling a continuous update.

CONCLUSION

An overview is given on the reported range of concentration of uremic retention solutes. Most known uremic retention solutes have a low molecular weight, although these not necessarily exert toxicity, especially if they are not protein bound. Concentrations of 90 solutes are reported, ranging from 2.3 g/L (urea) to 0.32 ng/L (methionine-enkephalin). Low concentrations are found especially for the middle molecules. The ratio C_U/C_N is high for several of the guanidines, protein-bound molecules, and middle molecules, and many of these solutes are toxic. These data can be of use as a guideline for future in vivo and in vitro experiments. Uremic retention appears to be a complex kinetic and multifactorial problem concerning a larger amount of solutes than those currently used as markers, such as urea and creatinine.

ACKNOWLEDGMENTS

The European Uremic Toxin Work Group (EUTox) has been created within the European Society for Artificial Organs (ESAO) to discuss and analyze matters related to the identification, characterization, analytic determination, and evaluation of biologic activity of uremic retention solutes. More information about the European Uremic Toxin Work Group, which is responsible for this publication, can be obtained at the website: <http://uremic-toxins.org>.

The authors would appreciate being contacted about uremic toxins or concentrations that are not listed in this publication. Please use the following e-mail address: raymond.vanholder@rug.ac.be.

Reprint requests to Raymond Vanholder, Nephrology Section, Department of Internal Medicine, University Hospital, De Pintelaan, 185, B9000. Ghent, Belgium.
E-mail: raymond.vanholder@rug.ac.be

REFERENCES

1. VANHOLDER R, DE SMET R: Pathophysiologic effects of uremic retention solutes. *J Am Soc Nephrol* 10:1815–1823, 1999
2. VANHOLDER R, DE SMET R, HSU C, et al: Uremic toxicity: The middle molecule hypothesis revisited. *Semin Nephrol* 14:205–218, 1994
3. VANHOLDER R, ARGILES A, BAURMEISTER U, et al: Uremic toxicity: Present state of the art. *Int J Artif Organs* 24:695–725, 2001
4. LEYPOLDT JK, CHEUNG AK, CARROLL CE, et al: Effect of dialysis membranes and middle molecule removal on chronic hemodialysis patient survival. *Am J Kidney Dis* 33:349–355, 1999
5. VANHOLDER R, DE SMET R, LAMEIRE NH: Redesigning the map of uremic toxins. *Contrib Nephrol* 133:42–70, 2001

6. BABB AL, AHMAD S, BERGSTROM J, SCRIBNER BH: The middle molecule hypothesis in perspective. *Am J Kidney Dis* 1:46–50, 1981
7. VANHOLDER R, CORNELIS R, DHONDT A, LAMEIRE N: The role of trace elements in uraemic toxicity. *Nephrol Dial Transplant* 17(Suppl 2):2–8, 2002
8. BLOCK GA, PORT FK: Re-evaluation of risks associated with hyperphosphatemia and hyperparathyroidism in dialysis patients: Recommendations for a change in management. *Am J Kidney Dis* 35:1226–1237, 2000
9. BLOCK GA, HULBERT-SHEARON TE, LEVIN NW, PORT FK: Association of serum phosphorus and calcium x phosphate product with mortality risk in chronic hemodialysis patients: A national study. *Am J Kidney Dis* 31:607–617, 1998
10. HENLE T, DEPPISCH R, BECK W, et al: Advanced glycosylated end-products (AGE) during haemodialysis treatment: Discrepant results with different methodologies reflecting the heterogeneity of AGE compounds. *Nephrol Dial Transplant* 14:1968–1975, 1999
11. WEISS MF, ERHARD P, KADER-ATTIA FA, et al: Mechanisms for the formation of glycoxidation products in end-stage renal disease. *Kidney Int* 57:2571–2585, 2000
12. NIWA T, TAKEDA N, YOSHIZUMI H: RNA metabolism in uremic patients: Accumulation of modified ribonucleosides in uremic serum. Technical note. *Kidney Int* 53:1801–1806, 1998
13. KANG ES, TEVLIN MT, WANG YB, et al: Hemodialysis hypotension: Interaction of inhibitors, iNOS, and the interdialytic period. *Am J Med Sci* 317:9–21, 1999
14. VALLANCE P, LEONE A, CALVER A, et al: Accumulation of an endogenous inhibitor of nitric oxide synthesis in chronic renal failure. *Lancet* 339:572–575, 1992
15. MARESCAU B, NAGELS G, POSSEMIERS I, et al: Guanidino compounds in serum and urine of nondialyzed patients with chronic renal insufficiency. *Metabolism* 46:1024–1031, 1997
16. DE DEYN PP, ROBITAILE P, VANASSE M, et al: Serum guanidino compound levels in uremic pediatric patients treated with hemodialysis or continuous cycle peritoneal dialysis. Correlations between nerve conduction velocities and altered guanidino compound concentrations. *Nephron* 69:411–417, 1995
17. TANAKA A, TAKAHASHI Y, MIZOKUCHI M, et al: Plasma, urinary, and erythrocyte concentrations of guanidino compounds in patients with chronic renal failure. *Ren Fail* 21:499–514, 1999
18. NIWA T, YAMAMOTO N, MAEDA K, et al: Gas chromatographic-mass spectrometric analysis of polyols in urine and serum of uremic patients. Identification of new deoxyalditols and inositol isomers. *J Chromatogr* 277:25–39, 1983
19. ROBOZ J, KAPPATOS D, HOLLAND JF: Polyol concentrations in serum during hemodialysis. *Clin Chem* 36:2082–2086, 1990
20. NIWA T, MAEDA K, OHKI T, et al: A gas chromatographic-mass spectrometric analysis for phenols in uremic serum. *Clin Chim Acta* 110:51–57, 1981
21. DE DEYN P, MARESCAU B, LORNOY W, et al: Guanidino compounds in uraemic dialysed patients. *Clin Chim Acta* 157:143–150, 1986
22. HEGBRANT J, THYSELL H, EKMAN R: Elevated plasma levels of opioid peptides and delta sleep-inducing peptide but not of corticotropin-releasing hormone in patients receiving chronic hemodialysis. *Blood Purif* 9:188–194, 1991
23. LOWRIE EG, LEW NL: Death risk in hemodialysis patients: the predictive value of commonly measured variables and an evaluation of death rate differences between facilities. *Am J Kidney Dis* 15:458–482, 1990
24. WENGLE B, HELLSTROM K: Volatile phenols in serum of uraemic patients. *Clin Sci* 43:493–498, 1972
25. GERRITS GP, MONNENS LA, DE ABREU RA, et al: Disturbances of cerebral purine and pyrimidine metabolism in young children with chronic renal failure. *Nephron* 58:310–314, 1991
26. MCGREGOR DO, DELLOW WJ, LEVER M, et al: Dimethylglycine accumulates in uremia and predicts elevated plasma homocysteine concentrations. *Kidney Int* 59:2267–2272, 2001
27. DE DEYN P, MARESCAU B, LORNOY W, et al: Serum guanidino compound levels and the influence of a single hemodialysis in uremic patients undergoing maintenance hemodialysis. *Nephron* 45:291–295, 1987
28. VANHOLDER RC, DE SMET RV, RINGOIR SM: Assessment of urea and other uremic markers for quantification of dialysis efficacy. *Clin Chem* 38:1429–1436, 1992
29. KOCK R, DELVOUX B, SIGMUND M, GREILING H: A comparative study of the concentrations of hypoxanthine, xanthine, uric acid and allantoin in the peripheral blood of normals and patients with acute myocardial infarction and other ischaemic diseases. *Eur J Clin Chem Clin Biochem* 32:837–842, 1994
30. BAYES B, PASTOR MC, BONAL J, et al: Homocysteine and lipid peroxidation in haemodialysis: Role of folic acid and vitamin E. *Nephrol Dial Transplant* 16:2172–2175, 2001
31. DANIEWSKA-MICHALSKA D, MOTYL T, GELLERT R, et al: Efficiency of hemodialysis of pyrimidine compounds in patients with chronic renal failure. *Nephron* 64:193–197, 1993
32. MARANGELLA M, PETRARULO M, MANDOLFO S, et al: Plasma profiles and dialysis kinetics of oxalate in patients receiving hemodialysis. *Nephron* 60:74–80, 1992
33. ZIMMERMAN L, JORNVAL H, BERGSTROM J: Phenylacetylglutamine and hippuric acid in uremic and healthy subjects. *Nephron* 55:265–271, 1990
34. NIWA T, KATSUZAKI T, MOMOI T, et al: Modification of beta₂-m with advanced glycation end products as observed in dialysis-related amyloidosis by 3-DG accumulating in uremic serum. *Kidney Int* 49:861–867, 1996
35. NIWA T, ISE M: Indoxyl sulfate, a circulating uremic toxin, stimulates the progression of glomerular sclerosis. *J Lab Clin Med* 124:96–104, 1994
36. ODANI H, SHINZATO T, MATSUMOTO Y, et al: Increase in three alpha,beta-dicarbonyl compound levels in human uremic plasma: Specific in vivo determination of intermediates in advanced Maillard reaction. *Biochem Biophys Res Commun* 256:89–93, 1999
37. FARRELL PC, GOTCH FA, PETERS JH, et al: Binding of hippurate in normal plasma and in uremic plasma pre- and postdialysis. *Nephron* 20:40–46, 1978
38. BOSTOM AG, SHEMIN D, LAPANE KL, et al: Hyperhomocysteinemia and traditional cardiovascular disease risk factors in end-stage renal disease patients on dialysis: A case-control study. *Atherosclerosis* 114:93–103, 1995
39. BRULEZ HF, VAN GULDENER C, DONKER AJ, TER WEE PM: The impact of an amino acid-based peritoneal dialysis fluid on plasma total homocysteine levels, lipid profile and body fat mass. *Nephrol Dial Transplant* 14:154–159, 1999
40. VAN GULDENER C, LAMBERT J, TER WEE PM, et al: Carotid artery stiffness in patients with end-stage renal disease: No effect of long-term homocysteine-lowering therapy. *Clin Nephrol* 53:33–41, 2000
41. LUDWIG GD, SENESKY D, BLUEMLE LW, JR, ELKINTON JR: Indoles in uremia: Identification by countercurrent distribution and paper chromatography. *Am J Clin Nutr* 21:436–450, 1968
42. ITAKA M, KAWASAKI S, SAKURAI S, et al: Serum substances that interfere with thyroid hormone assays in patients with chronic renal failure. *Clin Endocrinol* 48:739–746, 1998
43. SAITO K, FUJIGAKI S, HEYES MP, et al: Mechanism of increases in L-kynurenine and quinolinic acid in renal insufficiency. *Am J Physiol Renal Physiol* 279:F565–F572, 2000
44. SWAN JS, KRAGTEN EY, VEENING H: Liquid-chromatographic study of fluorescent materials in uremic fluids. *Clin Chem* 29:1082–1084, 1983
45. HEIMBURGER O, LONNQVIST F, DANIELSSON A, et al: Serum immunoreactive leptin concentration and its relation to the body fat content in chronic renal failure. *J Am Soc Nephrol* 8:1423–1430, 1997
46. DAGOGO-JACK S, OVALLE F, LANDT M, et al: Hyperleptinemia in patients with end-stage renal disease undergoing continuous ambulatory peritoneal dialysis. *Perit Dial Int* 18:34–40, 1998
47. VILJOEN M, STEYN ME, VAN RENSBUUR BW, REINACH SG: Melatonin in chronic renal failure. *Nephron* 60:138–143, 1992
48. HIDA M, AIBA Y, SAWAMURA S, et al: Inhibition of the accumulation of uremic toxins in the blood and their precursors in the feces after oral administration of Lebein, a lactic acid bacteria preparation, to uremic patients undergoing hemodialysis. *Nephron* 74:349–355, 1996
49. TANEDA S, MONNIER VM: ELISA of pentosidine, an advanced glycation end product, in biological specimens. *Clin Chem* 40:1766–1773, 1994

50. JANKOWSKI J, TEPEL M, STEPHAN N, *et al*: Characterization of p-hydroxy-hippuric acid as an inhibitor of Ca²⁺-ATPase in end-stage renal failure. *Kidney Int* 59(Suppl 78):S84-S88, 2001
51. SAITO A, TAKAGI T, CHUNG TG, OHTA K: Serum levels of polyamines in patients with chronic renal failure. *Kidney Int* 24(Suppl 16):S234-S237, 1983
52. NIWA T, YOSHIZUMI H, EMOTO Y, *et al*: Accumulation of quinolinic acid in uremic serum and its removal by hemodialysis. *Clin Chem* 37:159-161, 1991
53. KABANDA A, JADOUL M, POCHET JM, *et al*: Determinants of the serum concentrations of low molecular weight proteins in patients on maintenance hemodialysis. *Kidney Int* 45:1689-1696, 1994
54. ISHIMITSU T, NISHIKIMI T, SAITO Y, *et al*: Plasma levels of adrenomedullin, a newly identified hypotensive peptide, in patients with hypertension and renal failure. *J Clin Invest* 94:2158-2161, 1994
55. DERAY G, CARAYON A, MAISTRE G, *et al*: Endothelin in chronic renal failure. *Nephrol Dial Transplant* 7:300-305, 1992
56. STEIN G, FRANKE S, MAHIOUT A, *et al*: Influence of dialysis modalities on serum AGE levels in end-stage renal disease patients. *Nephrol Dial Transplant* 16:999-1008, 2001
57. AGUILERA A, CODOCEO R, SELGAS R, *et al*: Anorexigen (TNF-alpha, cholecystokinin) and orexigen (neuropeptide Y) plasma levels in peritoneal dialysis (PD) patients: Their relationship with nutritional parameters. *Nephrol Dial Transplant* 13:1476-1483, 1998
58. PASCUAL M, STEIGER G, ESTREICHER J, *et al*: Metabolism of complement factor D in renal failure. *Kidney Int* 34:529-536, 1988
59. SHIMOYAMA S, YAMASAKI K, KAWAHARA M, KAMINISHI M: Increased serum angiogenin concentration in colorectal cancer is correlated with cancer progression. *Clin Cancer Res* 5:1125-1130, 1999
60. HEGBRANT J, THYSELL H, EKMEN R: Erythropoietin treatment and plasma levels of corticotropin-releasing hormone, delta sleep-inducing peptide and opioid peptides in hemodialysis patients. *Scand J Urol Nephrol* 26:393-396, 1992
61. DE MEDINA M, ASHBY M, DIEGO J, *et al*: Factors that influence serum hyaluronan levels in hemodialysis patients. *ASAIO J* 45:428-430, 1999
62. PEREIRA BJ, SHAPIRO L, KING AJ, *et al*: Plasma levels of IL-1 beta, TNF alpha and their specific inhibitors in undialyzed chronic renal failure, CAPD and hemodialysis patients. *Kidney Int* 45:890-896, 1994
63. KIMMEL PL, PHILLIPS TM, SIMMENS SJ, *et al*: Immunologic function and survival in hemodialysis patients. *Kidney Int* 54:236-244, 1998
64. COHEN G, RUDNICKI M, SCHMALDIENST S, HORL WH: Effect of dialysis on serum/plasma levels of free immunoglobulin light chains in end-stage renal disease patients. *Nephrol Dial Transplant* 17:879-883, 2002
65. TSUKAMOTO Y, HANAOKA M, MATSUO T, *et al*: Effect of 22-oxacalcitriol on bone histology of hemodialyzed patients with severe secondary hyperparathyroidism. *Am J Kidney Dis* 35:458-464, 2000
66. DESCAMPS-LATSCHA B, HERBELIN A, NGUYEN AT, *et al*: Balance between IL-1 beta, TNF-alpha, and their specific inhibitors in chronic renal failure and maintenance dialysis. Relationships with activation markers of T cells, B cells, and monocytes. *J Immunol* 154:882-892, 1995
67. KAIZU Y, KIMURA M, YONEYAMA T, *et al*: Interleukin-6 may mediate malnutrition in chronic hemodialysis patients. *Am J Kidney Dis* 31:93-100, 1998
68. ZOCCALI C, BODE-BOGER S, MALLAMACI F, *et al*: Plasma concentration of asymmetrical dimethylarginine and mortality in patients with end-stage renal disease: A prospective study. *Lancet* 358:2113-2117, 2001
69. MASSY ZA, BADER CA, CHEVALIER A, *et al*: Serum lipoprotein (a) levels in chronic renal failure and renal transplant patients. *J Nephrol* 7:229-236, 1994
70. GAULT MH, LONGERICH LL, PURCHASE L, *et al*: Comparison of Lp(a) concentrations and some potential effects in hemodialysis, CAPD, transplantation, and control groups, and review of the literature. *Nephron* 70:155-170, 1995
71. MUCCI I, HERCZ G, ULDALL R, *et al*: Control of serum phosphate without any phosphate binders in patients treated with nocturnal hemodialysis. *Kidney Int* 53:1399-1404, 1998
72. LESAFFER G, DE SMET R, LAMEIRE N, *et al*: Intradialytic removal of protein-bound uraemic toxins: Role of solute characteristics and of dialyser membrane. *Nephrol Dial Transplant* 15:50-57, 2000
73. LOCATELLI F, MASTRANGELO F, REDAELLI B, *et al*: Effects of different membranes and dialysis technologies on patient treatment tolerance and nutritional parameters. The Italian Cooperative Dialysis Study Group. *Kidney Int* 50:1293-1302, 1996
74. WIESHOLZER M, HARM F, HAUSER AC, *et al*: Inappropriately high plasma leptin levels in obese haemodialysis patients can be reduced by high flux haemodialysis and haemodiafiltration. *Clin Sci* 94:431-435, 1998
75. COYNE DW, DAGOGO-JACK S, KLEIN S, *et al*: High-flux dialysis lowers plasma leptin concentration in chronic dialysis patients. *Am J Kidney Dis* 32:1031-1035, 1998
76. LOCATELLI F, MARCELLI D, CONTE F, *et al*: Comparison of mortality in ESRD patients on convective and diffusive extracorporeal treatments. The Registro Lombardo Dialisi E Trapianto. *Kidney Int* 55:286-293, 1999
77. PORT FK, WOLFE RA, HULBERT-SHEARON TE, *et al*: Mortality risk by hemodialyzer reuse practice and dialyzer membrane characteristics: Results from the USRDS dialysis morbidity and mortality study. *Am J Kidney Dis* 37:276-286, 2001
78. BLOEMBERGEN WE, HAKIM RM, STANNARD DC, *et al*: Relationship of dialysis membrane and cause-specific mortality. *Am J Kidney Dis* 33:1-10, 1999
79. MITRA M: How to reduce mortality in hemodialysis patients still a puzzle. *JAMA* 287:2643-2644, 2002
80. SARNATSKAYA VV, IVANOV AI, NIKOLAEV VG, *et al*: Structure and binding properties of serum albumin in uremic patients at different periods of hemodialysis. *Artif Organs* 22:107-115, 1998
81. MINGRONE G, DE SMET R, GRECO AV, *et al*: Serum uremic toxins from patients with chronic renal failure displace the binding of L-tryptophan to human serum albumin. *Clin Chim Acta* 260:27-34, 1997
82. VANHOLDER R, VAN LANDSCHOOT N, DE SMET R, *et al*: Drug protein binding in chronic renal failure: evaluation of nine drugs. *Kidney Int* 33:996-1004, 1997
83. VANHOLDER R, HOEFLIGER N, DE SMET R, RINGOIR S: Extraction of protein bound ligands from azotemic sera: Comparison of 12 deproteinization methods. *Kidney Int* 41:1707-1712, 1992
84. JOHNSON WJ, HAGGE WW, WAGONER RD, *et al*: Effects of urea loading in patients with far-advanced renal failure. *Mayo Clin Proc* 47:21-29, 1972
85. DE SMET R, DAVID F, SANDRA P, *et al*: A sensitive HPLC method for the quantification of free and total p-cresol in patients with chronic renal failure. *Clin Chim Acta* 278:1-21, 1998
86. JANIK JE, CURTI BD, CONSIDINE RV, *et al*: Interleukin 1 alpha increases serum leptin concentrations in humans. *J Clin Endocrinol Metab* 82:3084-3086, 1997
87. KAYSEN GA: The microinflammatory state in uremia: Causes and potential consequences. *J Am Soc Nephrol* 12:1549-1557, 2001
88. STENVINKEL P, HEIMBURGER O, WANG T, *et al*: High serum hyaluronan indicates poor survival in renal replacement therapy. *Am J Kidney Dis* 34:1083-1088, 1999