Uremic Toxins, and Their Effect on Intermediary Metabolism

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In the late stages of chronic renal damage the functional mass of the kidney is reduced and there is progression to renal insufficiency, usually called uremia, in which all aspects of renal function are affected. The complexity of the biochemical aspects of the syndrome of uremia is a manifestation of the wide variety and nature of the individual disorders that contribute to the pathogenesis of the final clinical syndrome. One major feature is the retention of metabolic end products and their effects, as toxins, on intermediary metabolism. The retained end products, working singly or in combination, probably affect metabolic pathways by some modification of enzymic reactions. They act at the cell membrane level. Although "middle molecules" have been incriminated as uremic toxins, recent attention has also focused on trace elements—especially aluminum, which has been incriminated in the pathogenesis of two major disorders, osteomalacic dialysis osteodystrophy and dialysis encephalopathy.

Additional Keyphrases: renal function • "middle molecules" • aluminum • dialysis patients • osteodystrophy • encephalopathy • trace elements • kidney disease • hormones • chronic renal failure • uremia • lipoproteins

Besides being the dominant organs in the homeostatic control of the constitution of the internal environment, the kidneys are also major endocrine organs, secreting various hormones that act on the respective target tissues. The kidneys also indirectly influence other endocrine systems, by affecting the clearance and degradation of other hormones. In reviewing the biochemical aspects of chronic renal failure—with specific reference to toxins—one must have a clear concept of the role that the kidneys play as homeostatic organs. However, the complexity of the syndrome of uremia is due to the role played by the kidneys, not only as homeostatic but also as endocrine organs.

As homeostatic organs the kidneys are responsible for the volume and the ionic and molecular composition of the internal environment. The concept of an internal fluid environment in which the cells of the body live and function was introduced by Claude Bernard in 1878 (1). The vital role of the kidneys in maintaining the constitution of that environment was subsequently recognized by Peters in 1935 (2), who said: "the kidneys appear to serve as the ultimate guardians of the constitution of the internal environment."

Excretion of metabolic end products, a major homeostatic function of the kidneys, involves both glomerular ultrafiltration and renal tubular secretion. During the development of the nephron unit the tubular secretory systems evolved to excrete, into an aqueous environment, waste products of high relative molecular mass that could not escape from the body by the process of simple diffusion (3). Maintaining optimal conditions in the fluid environment of the cells is vitally important; the failure of the kidneys to perform this task is manifested in the clinical syndrome called uremia.

The term "uremia" was introduced in 1840 by Pierry and l'Héritier (4). In their treatise on alterations in the blood they proposed use of the general suffix "-emia" to denote the blood compartment and qualifying it with a specific prefix to denote the presence of an abnormality in that compartment. Thus in their terminology "uremia" literally means "urine in the blood," which reflects their view that the toxic manifestations of renal failure were a form of poisoning of the blood, a consequence of reabsorption of urine. Today, uremia is used clinically to describe a complex syndrome that has many interrelated features.

In the specific context of toxins, the word "uremia" is used to describe the state associated with the retention of nitrogenous metabolic end products and is characterized by an increased concentration of urea in the blood. Despite a considerable amount of time and effort devoted to a search for the uremic toxin, no one individual compound has, so far, been incriminated. It may well be that no one uremic toxin will ever be identified as the toxin. The clinical syndrome of uremia should be recognized as a composite problem, involving all of the body's systems and reflecting biochemical alterations in all aspects of the constitution of the internal environment. In this view the alterations that produce uremia would reflect not only accumulation of metabolic end products but also associated changes in water, electrolyte, and acid–base homeostasis; disturbances in endocrine and nutritional status; and associated abnormalities in metabolism of fat, carbohydrate, and protein.

High concentrations in serum of the end products of protein catabolism are generally considered to be a major feature of the uremic state; some of the intermediate breakdown products are also believed to accumulate and play a role in the development of toxicity. Organic substances known or reported to accumulate in uremic blood include: urea, creatinine, guanidines and related compounds, uric acid, creatine, certain amino acids, polypeptides, polyamines, cyanate, indican (indoles), hippuric acid, phenols and conjugates of phenol, phenolic and indolic acids and their conjugates, organic acids of the tricarboxylic acid cycle, aliphatic amines, guanidine bases, pseudouridine, acetoin and 2,3-butylen glycol, β-oxo-butyrate, glucuronic acid, carnitine, myo-inositol, "middle molecules," sulfates, and phosphates (5). Given the improvement in clinical symptoms in uremic patients on institution of adequate therapy with dialysis, and the associated decrease in the concentra-

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tions of these retained organic metabolic end products, there is no doubt that the retained metabolites do play an important role in the pathogenesis of the clinical syndrome of uremia. The retained organic metabolic products probably play a major role as toxins in the pathogenesis of uremia, either working singly or in combination.

In this review I will deal with those compounds that have been of major interest in the past as toxins or are currently of interest.

**Urea**

Among the specific compounds incriminated and investigated as uremic toxins, urea has received considerable attention. The increase in the urea concentration in blood is perhaps the most striking abnormality of the body fluids in renal failure, although not the most important functionally.

Urea is formed only in the liver and may be regarded as the end product of protein catabolism, whether the protein originates from the diet or the tissues. Patients with acute renal failure show a relatively good correlation between the severity of the illness and the concentration of blood urea nitrogen. In chronic renal failure, however, the concentration of creatinine in serum seems to be a better index of the severity of the degree of failure, particularly when the patient is on a low protein diet.

The role of urea in the pathogenesis of the clinical syndrome of uremia has been controversial. In 1827 an early clinical chemist, Dr. John Bostock, who did the chemical analyses for Dr. Richard Bright, reported that in patients with chronic renal failure the serum "was found to consist in part of an animal matter possessing peculiar properties which seemed to approach those of urea" (6). Although Bright recognized the markedly increased concentrations of urea in patients with chronic renal failure, it is interesting that he considered that it "may be but in part a cause of general derangement of the system" (7). As has been subsequently demonstrated, the administration of urea to normal subjects in amounts sufficient to raise their blood concentrations to values similar to those in patients with chronic renal failure is associated only with thirst and polyuria, and none of the other clinical manifestations of uremia. In patients with chronic renal failure, therefore, most of the available evidence supports the proposal that an increased urea concentration in blood does not itself have a major toxic effect. In support of this proposal, Merrill et al. (8) hemodialyzed chronic uremic patients against solutions with high urea concentrations and noted an excellent clinical response even though the blood urea concentration was unaltered.

At one time, a high urea concentration in the blood was implicated in the pathogenesis of the "dialysis disequilibrium syndrome." This syndrome is characterized by the development of headache, confusion, muscle twitching, progressing occasionally to convulsions, coma, and (rarely) death, either during or towards the end of an otherwise biochemically successful dialysis. In the mechanism proposed by Kennedy et al. (9), an osmotic gradient was created, during dialysis, between the brain tissues, the cerebrospinal fluid, and extracellular fluid compartments, such that water would shift into the first of these; this event would be associated with an increase in intracranial pressure, which would account for the features of the syndrome. Since then, the dialysis disequilibrium syndrome has been found to relate to changes in sodium concentration during dialysis (10) and can be prevented by the use of a high sodium concentration in the dialyzing fluid (11).

Although urea may not itself be a major toxin in patients with chronic renal failure, it is one of the retained metabolites known to act as enzyme inhibitors (see below).

**Creatinine**

An increase in creatinine concentration in the plasma is another diagnostic feature of chronic renal failure; and it is roughly correlated with the degree of failure. A formula has been proposed that allows for age and weight and predicts the endogenous creatinine clearance ratio from the serum creatinine concentration (12). Like urea, creatinine may not, per se, play a role as a uremic toxin. Its retention is of importance in the genesis of other toxic metabolites, a consequence of an alteration in its normal metabolic pathway. Creatinine metabolism and excretion is altered in patients with chronic renal failure. In normal individuals, the amount of creatinine excreted is chiefly influenced by lean body mass and diet. Patients with chronic renal failure excrete less creatinine in their urine than would normally be expected. The serum creatinine concentration increases correspondingly in chronic renal failure, although an increasing proportion reportedly is cleared by an extrarenal mechanism, and this accounts for the reduction in urine excretion (13). The proposed extrarenal clearance mechanism involves two potential pathways: recycling of creatinine to creatine, irreversible degradation to products other than creatine, or both. Any alteration in creatinine metabolism during chronic renal failure, with an extrarenal clearance route, is of particular interest in that it represents an adaptive change in a metabolic pathway in response to the long-term decrease in functional renal mass.

**Uric and Oxalic Acids**

Urate retention is one of the recognized biochemical features of chronic renal failure. The uric acid in serum increases in uremia, but correlates poorly with the increment in creatinine concentration (14). As overall renal function deteriorates, the excretion and clearance of uric acid by the functional renal remnant markedly increases (14). An increase in the tubular secretion of uric acid and incomplete reabsorption of the filtered fraction—which is normally almost completely reabsorbed—would account for the lack of correlation with the increase in creatinine concentration. Again: the changes in the tubular secretion and reabsorption of uric acid represent adaptations to the decrease in functional renal mass. These adaptive changes in the remaining nephrons of the chronically diseased kidney with respect to uric acid transport may be prompted by a uricosuric factor in uremic serum (15, 16). In addition to an adaptive change in the renal tubular handling of uric acid there is also evidence that uremic patients develop an alternative route for either the metabolism or the clearance of uric acid (17). The alternative route involves the extrarenal elimination by the process of uricolysis (18), which takes place entirely in the intestinal tract, is catalyzed by bacterial enzymes, and appears to become increasingly important as the plasma uric acid concentration increases. Here again, this induction of alternative routes for the handling of uric acid, either by variations in renal tubular handling or the process of uricolysis, is of interest because of what it tells us of biochemical adaptations in response to decreased functional renal mass.

Retention of oxalic acid is a recognized feature in patients with chronic renal failure (19), and it reportedly is associated with the deposition of oxalate crystals in the myocardium and renal tissues (20–22). Crystal deposition in these tissues could be of importance in the genesis of some of the clinical features of the syndrome of uremia. It is also of interest in the overall pathogenesis of the clinical syndrome of uremia that oxalic acid is among the retained metabolites that are
known to act as enzyme inhibitors—in this particular instance, lactate dehydrogenase (23).

Myoinositol

Myoinositol has a specific effect on nerve conduction. Some patients with chronic renal failure have a marked increase in serum myoinositol concentration and also fail to clear it at the normal rate after an oral load. Changes in myoinositol concentration and clearance rate could result from an impairment of the renal catabolism (24). Myoinositol is a precursor and constituent of a class of phospholipids, the phosphoinositides, whose metabolism has been linked with the functional activity of nerve. Rats with hypermyoinositolemia from oral loading reportedly have a significant decrease in the velocity of sciatic motor-nerve conduction (24). On the basis of these findings it was suggested that the abnormally high myoinositol concentrations found in uremic patients may be a factor in the development and progression of a polyneuropathy. Most of the evidence, however, would now support the view that, although hypermyoinositolemia may depress nerve conduction velocity (25) and its concentrations in serum are markedly increased in patients with chronic renal failure, there is no indication that myoinositol is the neurotoxin of uremia (28). This view was based on the fact that there was no correlation between nerve-conduction velocities and serum or cerebrospinal fluid myoinositol concentrations: there was also no correlation between electroencephalographic changes and myoinositol concentrations in either of these two compartments (26).

Guandines and Related Compounds

Guandines and related compounds have long been implicated as uremic toxins. Experimental animals, chronically intoxicated with methylguanidine, reportedly lose weight at a rate that suggests that it exerts a catabolic action (27). In these animals the late stages of intoxication with methylguanidine were associated with disturbances in the gastrointestinal tract, the cardiovascular system, the lungs, and the central and peripheral nervous systems. The potential importance of methylguanidine as a toxin in uremia is that it is a constituent of creatine (methylguanidoacetic acid) and its derivative, creatinine.

Although the concentrations of guandine, methylguanidine, and 1,1-dimethylguanidine in serum may not be markedly increased in uremic patients, the urinary excretion of methylguanidine is significantly increased (28, 29). There is also evidence that the rate at which methylguanidine is produced metabolically is increased in chronic renal failure and that this is associated with increased concentrations in the tissues (30). Thus it was proposed that methylguanidine retention plays a role in the pathogenesis of the uremic syndrome (31). If methylguanidine is a uremic toxin, it presumably exerts its toxic actions in association with a preferential distribution in the intracellular compartment.

Although some experimental studies suggest that methylguanidine is a uremic toxin, other evidence supports the proposal that its toxic role remains to be defined. This proposal is based on observations that high concentrations of methylguanidine caused no significant inhibition of oxygen uptake in vitro in tissue-respiration studies of slices of rat cerebral cortex, kidney, and liver (32). The concentrations of methylguanidine used in those studies were up to four times those in serum of patients with chronic renal failure. In studies of glucose uptake and utilization by preparations of rat diaphragm, in vitro methylguanidine had a small enhancing effect (33) rather than a toxic inhibitory action. Guandine and guanidinoacetic acid also have been studied individually and shown to have small enhancing effects on glucose uptake and utilization (33). Guanidinoacetic acid is a precursor of creatinine, and its concentration increases during chronic renal failure as a direct consequence of the decreased clearance that can be effected by whatever functional renal tissue is left. An increase in guanidinoacetic acid potentially leads to the transfer of the amine group of arginine to aspartate instead of glycine, with the formation of guanidinoacetic acid and ornithine.

The urinary excretion of guanidinoacetic acid increases in chronic renal failure (34), and the increase has been attributed to the development of an alternative pathway for the detoxication of ammonia and urea synthesis, which involves guanidinoacetic acid. The mechanism for this alternative pathway would involve repression of normal enzyme activity and either the activation of a dormant enzyme or the appearance of a new enzyme. The alternative metabolic pathway for urea synthesis in chronic renal failure (35) is consistent with an increase in methylguanidine concentration. In subsequent studies Stein et al. (36) reported increased concentrations of guanidinoacetic acid in serum and cerebrospinal fluid and confirmed its high urinary excretion in patients with chronic renal failure. These observations have been confirmed by other workers, who reported that not only does guanidinoacetic acid accumulate in chronic renal failure, there was also evidence of increased production in these patients as compared with normal individuals (37). However, the role of guanidinoacetic acid as a toxin in uremia remains to be clarified; animal experiments gave (38) no evidence of guanidinoacetic acid being a uremic toxin.

Dimethylamine

In any review of the metabolites retained in the serum of patients with chronic renal failure it should be recognized that not all these compounds are derived either from a uremia-induced derangement of the usual metabolic pathway or from endogenous tissue sources. An example is dimethylamine, which increases in serum in uremia. Its concentration in duodenal contents is also increased as compared either with normal healthy subjects or with other types of patients (39). This increase is attributable to the uremic state per se, a consequence of uremia-induced alterations in the bacterial flora of the gastrointestinal tract. In uremic patients, part of the choline is transformed by bacteria in the gut to trimethylamine, which is reabsorbed and then either oxidized by trimethylamine dehydrogenase (EC 1.5.99.7) or demethylated to dimethylamine (DMA) in the liver (40). Dimethylamine enters the circulation and is excreted in bile and urine. The increased concentrations of dimethylamine and trimethylamine in the breath of uremic patients may be the classic "fishy odor" (41). Possibly, alterations in the gastrointestinal flora as a direct consequence of the uremic state are of importance in the formation of various other metabolites, which then are absorbed into the extracellular fluid compartment, resulting in an increased circulating concentration of them, with potential toxic and nutritional sequelae.

Parathryrin (Parathyroid Hormone) as a Uremic Toxin

Normally functioning kidneys play a major role in the enzymic degradation and clearance of hormones from the circulation, and because of this the kidneys may be regarded as indirect endocrine organs. Hormone status is disturbed in patients with chronic renal failure, and the associated consequences may be regarded as features of the clinical syndrome of uremia. An established biochemical feature of uremia is a markedly increased concentration of carboxy-
terminal immunoreactive parathyroid hormone (i-PTH), which not only reflects a disturbance in the rate of secretion of parathyrin but also a decrease, or even a failure, in the renal excretion of the peptide fractions resulting from enzymatic degradation. This accumulation of i-PTH degradation products, together with the known actions of the hormone, has led to the proposal that it may be a major uremic toxin.

In 1977, Massry (42) proposed that many of the manifestations of the uremic state could be accounted for by an excess of circulating parathyrin and that this polypeptide could be an important uremic toxin. The manifestations of uremia that were attributed to parathyrin included disorders of the central nervous system, soft-tissue calcification, soft-tissue necrosis, bone disease, pruritis, hyperlipidemia, anemia, and sexual dysfunction (43). The major potential role of parathyrin as a uremic toxin is as a neurotoxin, and a considerable amount of evidence supports this view. Avram et al. (44) reported a significantly decreased motor-nerve conduction velocity in uremic patients with above-normal i-PTH concentrations in their serum as compared with age-matched uremic patients with normal or only slightly above-normal i-PTH. There were no significant differences, in these two groups of patients, between the mean values for calcium and creatinine in serum. In subsequent studies the same workers added further support for the role of parathyrin as a neurotoxin with their observations that, after parathyroidectomy, motor nerve conduction velocity increased in a group of uremic patients on maintenance hemodialysis (45).

In experimental animals, an excess of parathyrin reportedly increases peripheral nerve calcium with a simultaneous decrease in motor-nerve conduction velocity (46), and these changes were reversed when the administration of parathyrin was stopped. Marked abnormalities in electroencephalographic patterns have been found in patients with acute renal failure who have concurrent increases in i-PTH; it was proposed that the abnormalities might be ascribed to a direct effect of parathyrin on the brain, causing an increase in calcium content (47). The electroencephalographic abnormalities show a direct relationship with the i-PTH values (48). In these studies, however, the significant correlation was with the values for the amino-terminal i-PTH fragment, and not with the carboxy-terminal fragment, in serum. Moreover, after six months of treatment with 1,25-dihydroxycholecalciferol, there was a marked decline in the concentrations of the amino-terminal i-PTH fragment in serum, which was associated with a significant change in the electroencephalogram toward normal in some of the patients. Thus, the currently available evidence would support the concept that parathyrin or its immunoreactive degradation products, or both, act as a neurotoxin in uremia.

An anemia of the normochromic normocytic type is almost always a feature of chronic renal failure. The kidneys play a major role in erythropoiesis and in the incorporation of iron into erythrocytes, by their production of erythropoietin. Nevertheless, the anemia of chronic renal failure has a multifactorial etiology; it is not simply the end result of a decrease in the renal production of erythropoietin. The pathogenesis of the anemia of chronic renal failure involves multiple factors, parathyrin among them. It is, however, important to recognize that among these the uremic state per se is of major importance; uremic serum has been recently reported to be toxic and inhibitory to erythropoiesis (52). The suppressive activity against erythroid colony growth in uremic serum appeared to be contained in compounds with molecular masses ranging from 47 000 to >150 000 Da. The importance of these observations is that inhibitors in this molecular-mass range would not be removed by conventional dialysis techniques and would not be classified as “middle molecules” (see below). An anemia of the normochromic normocytic type is a recognized clinical feature of patients with primary hyperparathyroidism. Although the anemia of chronic renal failure is of a different type, secondary hyperparathyroidism, with an excess of i-PTH, has been implicated as a factor in the pathogenesis of the anemia found in these patients (42). In uremic patients, subtotal parathyroidectomy, with a resulting decrease in serum i-PTH, is followed by significantly improved hemato logical indices (49–51). There is no evidence that an excess of parathyrin has a direct toxic effect on erythropoiesis. Rather, the improvement in the anemia after parathyroidectomy in uremic patients seems to be related to a decrease in the amount of fibrous tissue in their bone marrow—an increase in which is known to be induced by an excess of parathyrin.

### Middle Molecules

Most investigations into the pathophysiology of uremia have been directed towards the search for and isolation of either a single toxic substance or a group of substances, the retention of which would account for all of the clinical features of the syndrome. The most recent development in this search has been termed the “middle molecule” hypothesis, a concept that has developed as the direct consequence of the failure to incriminate such small molecules as urea and creatinine.

The middle molecule hypothesis is that a group of unknown compounds with molecular masses in the range of 300 to 1500 Da are of importance as toxic metabolites in uremia at relatively low concentrations (53, 54). The hypothesis is based on (a) a comparison of the clinical results obtained on using either hemodialysis or peritoneal dialysis and, more specifically, (b) the relationship between the number of hours of dialysis and the development of the symptoms of neuropathy. The assumption underlying the development of the middle molecule hypothesis is based on the difference between the molecular permeability of the cellulose membranes used in hemodialysis equipment as compared with that of the peritoneal membrane. The latter, which is known to leak protein, would presumably allow the removal of retained toxic metabolites with a higher molecular weight than those removed by dialysis across a cellulose membrane. The removal of toxic compounds of higher molecular mass would account for the better clinical results, as assessed by the alleviation of the symptoms of uremia, obtained with the peritoneal dialysis technique. A major feature of the middle molecule hypothesis is the concept that these unknown molecules exert their toxic effects at relatively low concentrations. Reportedly, middle molecules only accumulate in quantities that are sufficient to be measured in patients with advanced chronic renal failure, as assessed by an endogenous creatinine clearance rate of less than 11 mL/min (55).

Several recent studies have been aimed at identifying the middle molecules. One procedure revealed up to 10 identifiable subpeaks in the middle-molecule range in uremic sera, urine, and erythrocyte hemolysates (56). The technique used was a modification of a two-stage chromatographic procedure, involving use of a molecular sieve followed by ion-exchange chromatography (57). Differences were seen in the distribution of middle molecules among the three different materials studied (56). Although the concentrations of the middle molecules were increased in the serum of patients with uremia, the values for erythrocytes of patients with the syndrome were similar to those for normal subjects.
Thus Chapman et al. (56) concluded that "caution is necessary in interpreting results of middle molecule analyses." One biochemical aspect of their findings to which they drew attention is that if any of the species they identified is subsequently shown to be a uremic toxin, the differences in their distribution among the compartments of the body are of potential importance in the choice of an appropriate mathematical model for optimizing dialysis therapy.

Compounds in the middle-molecule range are said to be in higher concentrations in the serum of "sick" uremic patients than in an asymptomatic group of uremics (58, 59), although there is a considerable overlap between the data on individual middle-molecule fractions for the two groups (60). These authors could not correlate the accumulation of any specific middle-molecule fraction with the occurrence of any specific uremic symptom in the "sick" group of patients. In contrast, using a two-stage chromatographic technique—gel permeation followed by anion-exchange chromatography—one group of workers (61) has correlated the amplitude of one specific middle-molecule peak with the occurrence of uremic nephropathy. After successful renal transplantation, uremic middle molecules rapidly disappear from serum, even more rapidly than the creatinine concentration declines (62). Collectively these observations add support to the concept that compounds in the middle-molecule range may indeed play a role as uremic toxins.

A major problem in the development and understanding of the potential toxic nature of middle molecules has been the failure of various attempts to define both their chemical and biological nature. Menyhért and Gröf (63) proposed that a large group of unknown peptides with limited diffusibility through hemodialysis membranes constituted most of the uremic middle molecules. They investigated the molecular composition of uremic middle molecules in the 500-5000 range of relative molecular mass. Using cation-exchange column chromatography, they separated three fractions of serum containing these molecules. Subsequent one-dimensional paper chromatography of these fractions demonstrated that each of them could be further resolved into at least seven ninhydrin-positive subfractions. The qualitative amino acid composition of the subfractions differed from each other and from those of known peptides with which they were compared. Some of these peptides appeared to be completely removed from uremic serum by hemodialysis; others were only partly removed or were completely unaltered by this treatment.

The kidneys play a major role in the enzymatic degradation and clearance of hormones from the circulation. A disturbance in the normal clearance patterns of hormones potentially could play a role in the pathogenesis of the uremic syndrome. According to this concept, retention of either the hormones themselves or of their subsequently renal-cleared peptide degradation products could be regarded as potential uremic toxins, the unknown peptides reported by Menyhért and Gröf (63).

Although the clinical evidence I have mentioned supports the middle-molecule hypothesis, in the pathogenesis of the clinical syndrome of uremia their precise role still remains to be clarified. The middle-molecule hypothesis currently is used to refer to serum solutes in the relative molecular mass range of 500 to 2000 Da. In a recent study, uremic ultrafiltrates were fractionated by means of gel filtration and then further investigated by combined analytical techniques: "high-performance" liquid chromatography, liquid chromatography, gas chromatography, mass spectrometry, and isotachophoresis (64). These studies led the authors to propose that gel filtration was inappropriate as an analytical technique for determination of middle molecules, because they found that ultrafilterate fractions in the middle-molecular-mass range also contain a considerable amount of substances of low molecular mass, such as carbohydrates, organic acids, amino acids, and ultraviolet-absorbing solutes. The earlier work on middle molecules was largely based on gel filtration studies, so they recommended reconsideration of the importance of low-molecular-mass compounds and their role as uremic toxins. Other workers (65) have also concluded that "despite the extensive studies in this field, results are still confusing concerning the role of so-called 'middle molecules' in uremic toxicity." They (65) drew specific attention to the fact that a major problem in this field of investigation has been the differences between the analytical techniques used by different groups of workers, which has made it impossible to intercompare the reported middle-molecule fractions. They proposed that "it appears necessary to develop a chromatographic reference system which would be the basis for the evaluation of the in vitro and in vivo effects of the various molecules ranging in the still poorly defined M.W. fraction" (66). It can only be concluded at this time that, until the chemical identity of the retained uremic middle molecules is known and their biological toxicity has been demonstrated by experimental in vivo and in vitro studies, their role as uremic toxins is only hypothetical. Chemical identification and characterization of middle molecules will probably require mass spectrometry but this will have to await further technological advances in this field (66).

Retained Uremic Metabolites as Enzyme Inhibitors

A potential role of the retained serum metabolites as toxins in the pathogenesis of the uremic syndrome is as enzyme inhibitors. Urea, in concentrations comparable to those found in uremia, significantly inhibits monoamine oxidase (67). This enzyme has a major role in the destruction of both serotonin and catecholamines and in the regulation of amine metabolism in nervous tissue, and its inhibition by urea could be of significance in the pathogenesis of certain clinical features of the uremic syndrome. Urea also inhibits oxygen uptake by brain slices (68), but only at very high concentrations as compared with those in blood and after a 3-h lag period. Urea and creatinine reportedly inhibit glucose uptake and utilization in rat diaphragm in vitro; there was enhancement of the inhibitory effect when these two compounds were used together in the system (69). These latter findings are consistent with the proposal that the retained metabolites have a cumulative enzyme inhibitory effect in vivo.

Phenolic acids affect cerebral metabolism, as measured by the rate of respiration and anaerobic glycolysis of guinea pig brain slices, and they also inhibit the activity of some selected enzymes (70). The enzymes studied were the decarboxylases of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan, and glutamic acid; aspartate aminotransferase; 5'-nucleotidase; amine oxidase; and lactate dehydrogenase. Many aromatic acids, especially those with an unsaturated side chain, depressed enzyme reaction rates. The concentrations of phenolic acids used by Hicks et al. (70) were higher than those found in uremic serum, but they proposed that the lower concentrations of phenolic acids in uremic serum might possibly exert an effect by virtue of having been present for a longer time than the relatively high concentrations used in their enzyme inhibition studies. Alternatively, it could be that these compounds exert a cumulative inhibitory effect in vivo.

Aromatic compounds retained in uremia may exert an enzyme inhibitory action by a summation effect in vivo; both aromatic and aliphatic amines can cause enzyme inhibition
therefore amines (71). However, compared with the phenolic acids, the amines were less effective inhibitors of glutamic acid and dihydroxyphenylalanine decarboxylases. Amines cross the blood–brain barrier more readily than do acids (72) and therefore may be more effective in vivo than in vitro. The increased concentrations of aromatic amines in serum in uremia approximately correspond with the increase in blood urea nitrogen concentrations.

Although the precise biochemical role and the potential mechanisms of the retained metabolites as enzyme inhibitors in the etiology of the clinical syndrome of uremia remain to be clarified, these compounds clearly do accumulate in the extracellular-fluid compartment, and many of them can inhibit enzymes. Nevertheless, collectively they may exert their actions at a final common site. In one review of uremia, a unifying hypothesis for the toxicity of uremia was proposed, with derangements in membrane transport as the final common pathway (5). This hypothesis was based on a perspective of the wide range of known and potential toxic metabolites retained in uremic serum. Skeletal resistance to the action of exogenous parathyrin is a recognized feature of the disturbance in calcium homeostasis in patients with uremia. Wills and Jenkins (74), who studied parathyrin-induced bone resorption by using an in vivo organ culture system, reported that some known retained uremic metabolites, including phosphate, when tested individually, inhibited the calcium-mobilizing action of the hormone on bone, but the inhibitory effect of many of the individual metabolites was only significant at concentrations higher than those found in the serum of uremic patients. Serum collected from uremic patients prior to hemodialysis totally inhibited the calcium-mobilizing action of parathyrin; that collected from the same patients just after dialysis was not inhibitory. Thus they (74) proposed that if the uremic metabolites have an inhibitory effect on the action of parathyrin, it must be a cumulative phenomenon, which potentially could involve either the blockage of membrane receptors to parathyrin or hormone-induced effects on cell membrane transport.

Aluminum

The potential role of aluminum as a toxic agent in patients with normal renal function is controversial. Still, it has been established that in chronic renal failure there is an increased total body burden of aluminum and that this is associated with toxic sequelae involving bone, brain, and erythropoietic tissues (75). Aluminum salts are extensively used in the therapeutic management of the hyperphosphatemia that is a relatively constant feature of patients with chronic renal failure. Aluminum is absorbed from the gastrointestinal tract. In normal subjects, after an oral load its concentration in serum increases, followed by its increased excretion in the urine (76). Increased aluminum concentrations in the serum of some patients with chronic renal failure was first reported in 1970 (77). It is now established that hyperalumeminemia may occur in patients with end-stage chronic renal failure and is associated with the accumulation of aluminum in various tissues. Hyperalumeminemia and the associated toxic sequelae may occur in patients on treatment with either hemodialysis or peritoneal dialysis and in some patients who have not been dialyzed but are being treated with orally administered aluminum salts (75, 78). The high values for aluminum in serum and tissue result from the intestinal absorption of aluminum salts taken by mouth and from passage of aluminum across the dialysis membrane. The aluminum content of the dialyzing fluid obviously also depends on the Al content of the water used as the solvent. Some domestic tap-water contains aluminum in high concentration, either naturally or because aluminum has been added as a flocculant in the purification process. Acid rain markedly increases the “natural” aluminum content of water.

Aluminum is now recognized as a major, if not the major, toxic factor in the pathogenesis of a progressive fatal neurological syndrome in patients with chronic renal failure that was first reported in 1972 (79). The syndrome was later termed “dialysis encephalopathy” or “dialysis dementia,” and these patients showed increased amounts of aluminum in the brain, muscle, and bone tissue (80). Aluminum potentially exerts its neurotoxic action by inhibiting dihydropteridine reductase (EC 1.6.99.7) (81). Such inhibition would result in a decrease in the tetrahydrobiopterin, tyrosine, and neurotransmitters in brain. The neurotoxicity of aluminum may also involve alterations in the major post-synaptic enzymes of cholinergic neurotransmission (82). Although the precise mechanism possibly remains to be defined, much evidence indicates that aluminum is neurotoxic for patients whose functional renal mass is decreased. The latter leads to a reduction in the normal renal clearance of aluminum with a consequent increase in its concentration in serum and body tissue.

Bone pain, as a consequence of metabolic bone disease, is a common symptom in patients with chronic renal failure who are receiving long-term treatment with intermittent hemodialysis. The progressive metabolic bone disease in these patients is usually termed “dialysis osteodystrophy,” a term that distinguishes it, and some aspects of its pathogenesis, from renal osteodystrophy in the undialyzed patient. The osteomalacic component of dialysis osteodystrophy is a major clinical problem because it is associated with a high incidence of fractures. An increase in the Al content of bone in some patients with end-stage chronic renal failure was first reported in 1970 (83). Only years later was an association reported between the occurrence of dialysis encephalopathy and osteomalacia: exposure to high amounts of aluminum was a factor common to both of those complications in uremic patients (84, 85). Clearly, aluminum plays a major etiological toxic role in one particular type of osteomalacic dialysis osteodystrophy (75), a type that usually occurs in patients on dialysis treatment but may also occur in non-dialyzed patients (86). The mechanism for the disordered bone formation induced by an excess of aluminum remains to be clarified. It may involve a disturbance either in the formation of calcium apatite or in the bone-mineralization process (75, 87). There is also evidence from in vitro studies that suggests that aluminum may affect the activities of the bone enzymes, acid and alkaline phosphatase, and modify their response to parathyrin and 1,25-dihydroxycholecalciferol (88).

In patients on dialysis treatment who develop aluminum toxicity the major source of the aluminum is the tap-water used to prepare the dialysate. In addition there is also some intestinal absorption from the aluminum-containing phosphate-binding gels; in some patients this route appears to have been dominant. The driving force for aluminum transfer during dialysis appears to be the effective concentration gradient between the dialysate aluminum and the free diffusible aluminum fraction in serum (89). Transfer of aluminum from the dialyzing fluid across the dialyzing membrane appears to occur even when concentrations of the metal in the fluid are low (90, 91). The major portion, if not all, of aluminum in blood is bound to serum proteins and an as-yet-unidentified low-Mₙ species (91). The identification of the latter may be of importance in understanding the mechanisms of tissue accumulation and consequent toxicity.

Although aluminum has attracted much attention in recent years in this regard, it is important to recognize that
other trace metals in the serum and total body may be affected and may also play a role in the pathogenesis of some of the clinical features of uremia. Uremic patients show an increased nickel concentration in serum, but this does not appear to be associated with a corresponding increase in tissues, in contrast to the hyperaluminemia of chronic renal failure (92). As for other trace metals and their potential effects in chronic renal failure, it is important to recognize that deficiency states may also play a contributory role in the pathogenesis of the clinical syndrome of uremia. Zinc deficiency is a feature of uremia (93) and has been implicated as a major cause of the impairment in cellular immunity which is seen in these patients (94). The rates of sodium and potassium transport in vitro are normally influenced by the extracellular concentration of zinc; the cellular membrane transport of sodium reportedly is defective in leukocytes and erythrocytes from uremic patients. A low serum zinc concentration, however, does not account for the defect in membrane sodium transport in uremia (95), nor is it associated with an intracellular deficiency (95).

Conclusion

The biochemical disturbances—and in particular the role of the retained metabolites as toxins—in the pathogenesis of the syndrome of uremia are complex. The complexity is also manifested by the variety and nature of the individual disorders that make their individual contributions to the features seen in the final clinical syndrome. The complexity is a reflection of the importance of the homeostatic and endocrine roles of the kidneys in maintaining the constitution of the internal environment. In any review of uremia it is apparent that none of the body systems is spared. The constitution of the internal environment is disturbed not only by the retention of metabolic end products but also by the effects of the retained metabolites on intermediary metabolism. In addition to the many metabolites that are retained in the body fluids as a consequence of chronic renal failure there are major disturbances in total body electrolyte composition and the distribution of body water (96,97) and hormone-control mechanisms (98). Brennan et al. (96) reported that in patients with end-stage chronic renal failure there were significant increases in total body sodium, chloride, water, and extracellular fluid volume. These changes in total body water and sodium are linked both with alterations in their respective hormonal control mechanisms and with the clinical problem of hypertension in uremia. The role of the changes in extracellular fluid volume and electrolytes in the causation of some of the other biochemical and metabolic disturbances in the syndrome of uremia is not clearly defined.

The complexity, interactions, and consequences of the uremic state and the potential role of toxins are exemplified by a consideration of the disorder in carbohydrate and insulin metabolism in chronic renal failure. Patients with chronic renal failure have a diminished ability to handle a glucose load, the degree of glucose intolerance correlating roughly with the severity of the uremia (3). The disturbance in glucose metabolism is the end result of the summation of several interrelated factors, including toxins. The dominant feature in the disturbance would appear to be an impairment of the insulin-dependent transfer of glucose into extrahepatic tissues and its subsequent utilization, with a defect in insulin synthesis or release (or both) playing a relatively minor role. The primary defect in the sequence appears to be an insensitivity of peripheral tissue to insulin, probably a defect either in intracellular metabolism or in the glucose transport system (99). The importance of the disturbance in carbohydrate and glucose metabolism is not in any way the direct result of variations in blood glucose concentration—hyperglycemia is rare—rather, it is ascribable to the important etiological role it potentially plays in the disturbance in lipoprotein metabolism in uremia. The precise cause of the dyslipoproteinemia of chronic renal failure is not clear, although the disorder in carbohydrate metabolism is probably a major factor. The severity of the dyslipoproteinemia has shown no consistent correlation with the nature of the underlying renal disease, the degree or duration of renal failure, or the diet. The patterns of the disturbance in lipoprotein metabolism in patients with chronic renal failure take several forms: an increase in the concentration and cholesterol content of very-low-density lipoprotein; an increase in the size and triglyceride content of low-density lipoprotein particles; a decreased concentration of cholesterol in high-density lipoprotein; and an increased prevalence of an electrophoretic subclass (late pre-β-lipoprotein) of very-low-density lipoprotein (100,101). The importance of the dyslipoproteinemia of uremia is that it is related to the accelerated atherogenesis and premature coronary artery disease that is a clinical feature of patients with chronic renal failure, particularly those being treated by long-term intermittent hemodialysis.

Uremia is a multi-system clinical and biochemical problem that still requires extensive clarification. It would seem unlikely, in the final analysis, if any one individual toxin will ever be identified as "the" toxin; rather, the syndrome of uremia probably will be attributed to a cumulative effect in vivo, to which all of the retained metabolites contribute. In the process of evolution the kidneys have developed, during the past 600 million years, into complex organs, sharply in contrast to the simple open-ended tubes that drained waste products from the coelomic cavity of our early remote ancestors (3). As complex, sophisticated homeostatic and endocrine organs the kidneys play the dominant role in the maintenance of the internal environment. The failure to remove metabolic waste end products from the urine, their consequent retention in the blood and tissue compartments, and their potential cumulative action as toxins are major features in the pathogenesis of the clinical syndrome of uremia.

References

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