Biochemistry of Renal Failure

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ABSTRACT

The term uremia is used clinically to describe that state associated principally with the retention of nitrogenous metabolic products and is characterized by a raised blood urea concentration. The increase in the blood urea concentration is perhaps the most striking abnormality of the body fluids in renal failure, although it is not the most important functionally. The clinical syndrome of uremia involves all the systems of the body as a result of biochemical alterations in the constitution of the internal environment. The alterations in the latter compartment are due not only to the retained metabolic products but also to associated changes in water, electrolyte, and acid-base homeostasis. There are also features of the clinical syndrome of uremia which are attributable to variations in the rates of secretion and metabolism of a number of hormones in which the kidney is recognized to play a role, either directly or indirectly. This brief review deals with the retained uremic metabolites and their biochemical significance in the clinical syndrome of uremia. The metabolites discussed include urea, creatinine, guanidines and related compounds, uric acid, dimethylamine, and the middle molecule hypothesis. The potential role of some of these metabolites is discussed with regard to their potential toxic role as enzyme inhibitors. The biochemical aspects of uremic neuropathy are reviewed to highlight the biochemical complexity of the investigation of the uremic syndrome.

Introduction

The excretion of metabolic waste products is one of the main functions of the kidney. The processes involved in this excretory function are those of glomerular ultrafiltration and renal tubular secretion. The tubular secretory systems were originally evolved to excrete large molecular weight waste products that could not escape from the body, in an aqueous environment, by simple diffusion. Three types of renal secretory mechanism are involved in the transport of materials from the peri-tubular fluid to the tubular lumen. These include: (1) active secretory mechanisms which exhibit an absolute limitation of transport capacity (Tm-limited); (2) active secretory mechanisms which exhibit gradient-time limitation of
transport capacity; and (3) passive secretory mechanisms which involve diffusion down either a concentration or electrical potential gradient. It is generally agreed that there are three active secretory mechanisms which are Tm-limited and secrete organic compounds. One of these mechanisms secretes organic acids and another secretes a group of strong organic bases. These two mechanisms, which are discrete, are localized in the proximal tubule. Hydrogen ions are handled by an active, gradient-time limited transport mechanism throughout the length of the nephron, with some local variations in different sections of the tubule. The passive tubular secretory mechanisms are involved, mainly, in the excretion of both weak bases and acids. Passive diffusion occurs in all parts of the tubule but, as this mechanism is enhanced by hydrogen ion gradients, it is maximal in the distal part.

Uremia

Renal diseases in their early phases may affect primarily either the glomerulus or the renal tubules. In the later stages of chronic renal damage, the functional mass of the kidney is reduced and there is progression of renal insufficiency, usually called uremia (or occasionally azotemia), in which all aspects of renal function are affected. The term uremia means literally urine in the blood. The clinical manifestations of renal failure were originally regarded as a form of poisoning of the blood owing to the reabsorption of urine. The term is now used clinically to describe that state associated principally with the retention of nitrogenous metabolic products and the condition is characterized by a raised blood urea concentration; the increase does not necessarily correlate well with other aspects of renal insufficiency.

The increase in the blood urea concentration is perhaps the most striking abnormality of the body fluids in renal failure, although it is not the most important functionally. The retained organic metabolic products play a role in the pathogenesis of the syndrome either working singly or in combination to affect metabolic pathways by some modification of enzymatic reactions. The clinical syndrome of uremia, however, involves all the body’s systems as a result of biochemical alterations in the constitution of the internal environment. The alterations in the latter compartment are due not only to the retained metabolic products but also to the associated changes in water, electrolyte, and acid-base homeostasis. There are also features of the clinical syndrome of uremia which are attributable to variations in the rates of secretion and metabolism of a number of hormones in which the kidney is recognized to play a role, either directly or indirectly.

The overall biochemical disturbances found in patients with chronic renal failure and their metabolic consequences have been extensively reviewed recently elsewhere. This brief review will, therefore, deal only with the biochemical problems associated with the retained uremic metabolites and their biochemical significance in the clinical syndrome of uremia.

Urea

Urea is formed only in the liver and may be regarded as the end product of protein catabolism, whether the protein is derived from dietary or tissue sources. In patients with acute renal failure, there is a relatively good correlation between the severity of the illness and blood urea nitrogen concentration. In patients with chronic renal failure, the plasma creatinine concentration seems to be a better index of the severity of the degree of failure.

The role of urea in the pathogenesis of the clinical syndrome of uremia has been controversial. In normal subjects, the administration of urea, in amounts sufficient to raise their blood concentrations to values comparable to those found in patients
with chronic renal failure, is only associated with thirst and polyuria and none of the other clinical manifestations of uremia. In patients with chronic renal failure, the bulk of the available evidence would support the view that the increase in their blood urea concentration has of itself little or no major effect, other than those attributable to the osmotic diuresis which it causes. It is, however, recognized that urea is included amongst those retained metabolites that may act as enzyme inhibitors.

**Creatinine**

An increase in plasma creatinine concentration is a diagnostic feature of chronic renal failure and correlates very approximately with the degree of failure. It has been reported, however, that it is possible, with a formula that allows for age and weight, to predict the endogenous creatinine clearance rate from the serum creatinine concentration. In patients with chronic renal failure, the ratio of the serum concentration of urea nitrogen to creatinine has been reported to correlate closely with the dietary protein intake. The ratio can be used to indicate the presence of unrecognized catabolic stress or dehydration in the biochemical monitoring of chronic renal failure.

In patients with chronic renal failure, the increase in plasma creatinine is associated with a decrease in urinary excretion. In this group of patients, it has been reported that there is an alteration in creatinine metabolism which is a feature of the uremic state. In this situation, an increasing fraction of creatinine appears to be metabolized rather than excreted and two metabolic pathways are potentially involved; creatinine is either recycled to creatine or it is irreversibly degraded to other products.

**Guanidines and Related Compounds**

The toxic role of guanidine compounds was first reported in studies of experimental uremia by Mason et al in 1937. They concluded at that time, in view of problems in methodology for the determination of guanidine and its derivates, that it was "not justifiable to assign this substance a definite role in the pathogenesis" of the uremic syndrome. In experimental dogs chronically intoxicated with methylguanidine, it has been reported that there was a decrease in body weight at a rate which suggested that methylguanidine exerted a catabolic action. The late stages of intoxication with methylguanidine were associated with disturbances in the gastro-intestinal tract, the cardiovascular system, the lungs, and the central and peripheral nervous systems. In uremic patients, although the serum concentrations of guanidine, methylguanidine, and 1,1-dimethylguanidine may not be markedly increased, a significant increase has been reported in the urinary excretion of methylguanidine. There is also some evidence from animal experiments that the metabolic production rate of methylguanidine is increased in renal failure and that tissue concentrations are elevated. These observations have led to the view that methylguanidine retention occurs in chronic renal failure and plays a role in the etiology of the symptoms of the syndrome of uremia. The latter workers also proposed that uremic toxins, such as methylguanidine, may have a preferential distribution in the intracellular compartment.

**Uric Acid**

The retention of uric acid is one of the recognized biochemical features of chronic renal failure, although it is rarely associated with attacks of classical gout. The increase in plasma uric acid concentration in these patients correlates poorly with the creatinine concentration. The lack of correlation may be attributable to the observations that in patients with chronic renal failure, as total renal function deteriorates, there is a marked in-
crease in the excretion and clearance of uric acid in the functional renal remnant. These changes were reported to be due to an increase in the tubular secretion of urate and to incomplete reabsorption of the filtered fraction, which is normally almost completely reabsorbed. Danovitch and his colleagues proposed that these changes in the functional capacity of the remaining nephrons of the chronically diseased kidney, with respect to uric acid transport, might be due to a uricosuric factor in uremic plasma. In addition to an increase in the renal clearance of uric acid, there is also evidence that the extra-renal elimination is increased in chronic renal failure. The extra-renal elimination of uric acid 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.

Other Retained Metabolites

It is generally accepted that high plasma concentrations of the products of protein catabolism are a major feature of the uremic state and that some of the intermediate breakdown products accumulate and play a role in the development of the toxicity of the clinical syndrome of uremia. The organic substances known or reported to accumulate in uremic blood, in addition to urea, uric acid, and creatinine, include creatine, certain amino acids, polypeptides, indican, 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, acetoin and 2:3 butylen glycol, β-hydroxybutyrate, glucuronic acid, carnitine, myoinositol, oxalic acid, sulfates, and phosphates. It would seem probable that some of these substances diffuse into the brain and other tissues, and that the toxemia of chronic renal failure is due to a summation effect of these organic compounds, possibly including urea, acting as enzyme inhibitors.

The increased plasma concentrations of metabolites may not, however, be derived from endogenous tissue sources. An increased duodenal dimethylamine content has been reported in patients with uremia when compared with normal healthy subjects and with other groups of patients. It was suggested in these studies that the formation of such toxic metabolites in the small bowel may have significant nutritional and toxic sequelae in uremia. In uremia, choline is transformed in part, by bacteria in the gut, to trimethylamine (TMA) which is reabsorbed and then either oxidized by TMA oxidase or demethylated to dimethylamine (DMA) in the liver. Dimethylamine enters the circulation and is excreted in bile and urine. An increase in the breath concentrations of dimethylamine and trimethylamine in uremic patients is correlated with the classic "fishy odor" of the breath in the uremic state. It is possible that alterations in the bowel flora, as a consequence of the uremic state, are of importance in the formation of a variety of toxic metabolites which are then absorbed into the extracellular fluid compartment with a consequent rise in their circulating concentrations.

In recent years, attention has been directed towards the hypothesis that middle molecules are of importance as potential toxic metabolites in uremia. This hypothesis invokes as toxic metabolites a group of unknown compounds with molecular weights in the range of 300 to 1,500 which exert their toxic effects at relatively low concentrations. Using a two-stage chromatographic procedure, up to ten identifiable subpeaks in the middle molecule range have been observed in uremic sera, urine, and red cell hemolysates. The technique used in that study was a modification of a two-stage chromatographic procedure using a molecular sieve* followed by ion-exchange† chromatography. There is

* Sephadex G15.
† DEAE Sephadex A25.
evidence that the concentrations of compounds in the middle molecule range were higher in the plasma of a group of “sick” uremic patients when compared to an asymptomatic group. These findings support the hypothesis that these compounds may play a role as uremic toxins. The accumulation of any specific middle molecule fraction was not, however, correlated with the occurrence of a specific uremic symptom in the “sick” group of patients. The nature and identity of middle molecules and their role, if any, in the etiology of the clinical syndrome of uremia still remain to be clarified.

The kidney plays a major role in the enzymatic degradation and clearance of hormones from the circulation. It is possible that disturbances in normal hormone clearance patterns play a role in the pathogenesis of the uremic syndrome. Massry proposed that many of the manifestations of clinical syndrome of uremia could be accounted for by an excess of parathyroid hormone and that this polypeptide could be an important uremic toxin. The clinical manifestations of uremia that may be attributed to parathyroid hormone include: disorders of the central nervous system, soft tissue calcification, soft tissue necrosis, bone disease, pruritis, hyperlipidemia, and anemia and sexual dysfunction. Others have added evidence to support the hypothesis that parathyroid hormone is a neurotoxin. Their support was based on the observations that follow parathyroidectomy there was an increase in motor nerve conduction velocity in a group of uremic patients on maintenance hemodialysis. Further studies are needed to clarify the potential role of parathyroid hormone as a multi-system uremic toxin.

Retained Metabolites as Enzyme Inhibitors

The major toxic role of retained metabolites in the biochemical aspects of the syndrome of uremia would appear to be as enzyme inhibitors. Phenolic acids have been reported to have an effect on cerebral metabolism as measured by the rate of respiration and anaerobic glycolysis of guinea-pig brain slices and also to inhibit the activity of some selected enzymes involved in cerebral metabolism. The enzymes studied were the decarboxylases of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan and glutamic acid, glutamic oxaloacetic transaminase, 5'-nucleotidase, amine oxidase, and lactic dehydrognase. It was reported that many aromatic acids, especially those with an unsaturated side chain, depressed enzyme reaction rates. The phenolic acid concentrations used in those studies were higher than those found in uremic plasma, but it was proposed that the lower plasma concentrations of phenolic acids present in uremic patients might possibly exert an effect by virtue of being present for a longer time than the relatively high concentrations used in the enzyme inhibition studies.

It is also possible that retained aromatic compounds may exert an enzyme inhibitory action by a summation effect in vivo. Enzyme inhibition has been reported by aromatic and aliphatic amines; however, compared with the phenolic acids, the amines were less effective inhibitors of glutamic acid and di-hydroxyphenylalanine decarboxylases. Amines have been shown to cross the blood-brain barrier more readily than acids and the amines, therefore, may be more effective in vivo than in vitro. Morgan et al reported elevated plasma concentrations of aromatic amines in uremia and noted that the values corresponded roughly with the elevation in the blood urea nitrogen concentration. The precise biochemical role and the potential mechanisms of the retained metabolites as toxic factors in the etiology of the clinical syndrome remains to be clarified; there is, however, no doubt that these compounds do accumulate in the extracellular fluid compartment.
Uremic Neuropathy

A neuropathy, usually affecting the lower limbs, is one of the major clinical features of the syndrome of uremia, and the etiological mechanism has been investigated extensively biochemically in view of the nature of the lesions. The biochemical aspects of this neuropathy highlight the complexity of the investigation of the uremic syndrome. The motor symptoms of the neuropathy are predominantly of nocturnal cramps and "restless legs" with, occasionally, muscle weakness. Sensory complaints are less frequent and consist mainly of paraesthesia and dysoesthesia with, rarely, darting pains and a burning sensation in the feet. Vibrational sense is frequently impaired with loss of cutaneous sensibility. There is often a progressive diminution, with eventual loss, of ankle and knee jerks with, rarely, wasting of the distal muscle groups of the lower limb. The syndrome appears in a high proportion of patients with long-standing progressive chronic renal failure; males appear to be more susceptible than females. The nerve lesions have two components: an ascending segmental demyelination with axonal degeneration, and a toxic inhibition of nerve-membrane function. The etiology of this uremic neuropathy is unknown. It is, however, generally agreed that the major etiological factor is the accumulation of toxic metabolites. The nature of the toxic metabolites that cause the neuropathy have not been defined, although their effects have been linked with disturbances in nerve protein and phospholipid metabolism, and the maintenance of myelin.

On the basis of a microscopic, ultrastructural and biochemical study of nerve biopsies, it was proposed that the neuropathy of chronic renal disease was in part caused by disturbances in protein metabolism of the Schwann cells. Transketolase plays a role in the maintenance of myelin, and it has been suggested that the toxic metabolites in chronic renal failure may cause depression of transketolase activity leading to myelin sheath degeneration and the subsequent neuropathy. Lonergan and his colleagues reported that erythrocyte transketolase activity was depressed in plasma samples from chronic renal failure patients prior to hemodialysis and was significantly improved in post-dialysis specimens; they considered that their patients were not thiamine deficient. Other workers, however, have found normal transketolase activity in patients with chronic renal failure. Thomas proposed that the known but unexplained disturbance in pyruvate metabolism in uremic patients after a glucose load may offer a clue to the origin of uremic neuropathy. This proposal was based on the fact that this disturbance in pyruvate metabolism also occurs in thiamine deficiency; uremic neuropathy could, therefore, be due to some interference with thiamine metabolism. The latter would presumably be due to the uremic state per se.

In some patients with chronic renal failure, there is a marked increase in plasma myoinositol concentration, with a failure of normal clearance after an oral load; these changes could result primarily from impairment of renal myoinositol catabolism. Myoinositol is a precursor and constituent of a class of phospholipids, the phosphoinositides, whose metabolism has been linked with the functional activity of nerve. In experimental rats, Clements and his colleagues reported that the induction of hypermyoinositolemia with oral loading was followed by a significant decrease in sciatic motor-nerve conduction velocities. On the basis of these findings, it was suggested that in patients with chronic renal failure, the abnormally raised myoinositol concentrations may be the plasma factor that influences the development and progression of the polyneuropathy. The bulk of the evidence would now support the view that although hypermyo-
inositolemia may depress nerve conduction velocity and the plasma concentrations are markedly increased in chronic renal failure, there is no indication that myoinositol is a neurotoxin.

**Conclusion**

The nature of the fundamental biochemical disturbance or disturbances that cause the multi-system clinical syndrome of uremia are a major problem that requires clarification. There can be no doubt that the retained metabolites play a dominant toxic role. Although many metabolites have been identified and incriminated as "the" toxic factor in the causation of the uremic syndrome, the dominance of any one factor has yet to be established. It would seem most probable that in patients with chronic renal failure, no one individual metabolite will ever be incriminated but rather all exert a toxic cumulative effect in vivo acting as inhibitors of enzyme activity.

**References**

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