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Biochemical consequences of chronic renal failure: A review

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The biochemical consequences of chronic renal failure involve a disturbance in one of the fundamental mechanisms of the body's self-regulatory control systems. The concept of self-regulation in biological systems is both old and well established. The idea held by Hippocrates that disease was cured by natural powers, by a 'vis medicatrix naturae', implied the existence of systems ready to cooperate in a corrective manner when the natural state of the body was disturbed. In 1878 Claude Bernard made the distinction between the 'milieu extérieur', in which the whole organism exists, and the 'milieu intérieur', in which the cells live. After considering some of the mechanisms involved he wrote in his now classical sentence that: '. . . tous les mécanismes vitaux, quelque variés qu'ils soient, n'ont toujours qu'un but, celui de maintenir l'unité des conditions de la vie dans le milieu intérieur.' The concept of an internal environment that was maintained in a steady state was further developed during the next 50 years and the term homeostasis was introduced by Walter B. Cannon to describe the physiologically constant state of the body. In 1929 he wrote: 'The coordinated physiological reactions which maintain most of the steady states in the body are so complex, and so peculiar to the living organism, that it has been suggested (Cannon, 1925) that a specific designation for these states be employed—homeostasis'. The role of the kidney in homeostasis is perhaps best expressed in the sentence of J. P. Peters (1935) who wrote: 'The kidneys appear to serve as the ultimate guardians of the constitution of the internal environment.' The kidney carries out its homeostatic functions by the processes of glomerular filtration and of tubular reabsorption and secretion, and thus regulates the concentration of metabolic end products, the osmotic pressure, the volume and the ionic composition of the internal environment. In chronic renal failure, the end results of the disturbances of renal function are alterations in the constitution of the internal environment which are of fundamental biochemical significance.

UREA AND OTHER ORGANIC METABOLIC PRODUCTS

At their onset renal diseases may affect primarily the functions of either the glomerulus or the renal tubules, but in the latter stages of chronic renal damage the functional mass of the kidney is reduced and there is progression to renal insufficiency affecting all aspects of renal function, usually called 'uraemia'. The term 'uraemia', which was introduced in medical nomenclature in 1840 by Piorry and l'Héritier, means literally 'urine in the blood'. Piorry and l'Héritier regarded the manifestations of renal failure as a form of poisoning of the blood due to the resorption of urine. The term is now used clinically to describe that state associated with the retention of nitrogenous metabolic products and characterized by a raised blood urea concentration though the level 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. Urea is formed only in the liver and may be regarded as the end product of protein catabolism, whether the protein is derived from diet or tissues. In acute renal failure the correlation between the severity of the illness and the level of the blood urea concentration applies more closely than it does in chronic renal failure. In chronic renal failure the plasma creatinine concentration seems to offer a better index of the severity of the degree of failure than does the blood urea level, particularly when the patient is on a low-protein diet.

Bostock (1827a, b), who carried out the chemical examinations of serum from Richard Bright's patients, was the first to draw attention to the raised blood urea level found in chronic renal failure. He reported that the serum 'was found to consist in part of an animal matter possessing peculiar properties which seemed to approach those of urea'. Two years later Christison (1829) isolated urea from the blood of patients with chronic renal failure and

reported that in these patients 'urea exists in considerable quantity in the blood when it is materially defective in the urine'. The controversial role of urea in the causation of the clinical symptoms seen in chronic renal failure was first raised by Bright in 1831. He reported that in many cases the serum 'was slightly impregnated with urea' but in other cases this 'impregnation' was much less apparent and he concluded that the raised blood urea level 'may be but in part a cause of general derangement of the system'. Since then there have been conflicting reports and the position remains controversial. Pitts (1968) stated that when 'urea is administered to a normal person in amounts sufficient to achieve blood levels comparable to those found in chronic renal failure, it causes thirst and polyuria but none of the other manifestations of uraemia'. The position is, however, by no means as clear cut as this statement would suggest. In 1822 Ségalas d'Etchepare gave urea intravenously to dogs and reported that the only effect was to cause polyuria. Bollman and Mann (1927), also using dogs, reported that after ureteral transplantation into the jejunum or ileum, despite a marked elevation in the blood urea concentration, there were no symptoms of uraemia. In support of the view that a high blood urea level of itself has little or no effect is the work of Merrill, Legrain, and Hoigne (1953). These workers haemodialyzed chronic uraemic patients against high urea bath levels and noted that there was an excellent clinical response despite the lack of a change in the blood urea level. Other reports, however, ascribe a toxic role to urea itself at high blood levels. Hewlett, Gilbert, and Wickett (1916) gave urea by mouth to normal human subjects and raised their blood urea concentration to levels in the range from 160 to 245 mg/100 ml. The subjects noted that definite symptoms occurred at the time when the blood urea levels were at their highest. The symptoms they experienced consisted of 'headache, dizziness, apathy, drowsiness, bodily weakness, and fatigue'. Severe symptoms were reported by Grollman and Grollman (1959) at higher blood urea levels using dogs as their experimental animal. These workers studied bilaterally nephrectomized dogs who were maintained by intermittent peritoneal lavage. In one group the blood urea levels were maintained at high levels by using a dialysate with a high urea concentration. At blood urea levels of 370 to 480 mg/100 ml clinical symptoms of intoxication became apparent. The first symptoms noted were of weakness and anorexia, followed by vomiting and diarrhoea, the latter terminating in bowel haemorrhage; the symptoms finally culminated in coma. The control animals, on the same regimen except for the absence of urea from the dialysate, were maintained in apparently normal condition for several months. The only variable in their experiment was the high urea content of the extracellular fluid and they attributed the observed clinical abnormalities to this variable.

Although the elevation of blood urea concentration may or may not of itself cause symptoms, it is generally accepted that products of protein catabolism are a major feature of the uraemic state and that some of the intermediate breakdown products accumulate and play a role in the development of the toxicity of uraemia. The earlier workers searched for a single toxic substance to account for the clinical symptoms seen in uraemia. Foster (1915) reported the isolation of an organic base from the blood of uraemic patients which when injected into guinea pigs caused toxic symptoms within five minutes after injection. The initial symptoms were rapid breathing and muscular twitching followed by convulsions which terminated in death. The substance isolated was probably guanidine or a guanidine-like derivative (Harrison and Mason, 1937). During this period many other substances were implicated and included creatinine, potassium, 'nephrolysins', and phenols (Harrison and Mason, 1937). Mason, Resnik, Minot, Rainey, Pilcher, and Harrison (1937) discounted the so-called 'unitary' theories of uraemia because they had received neither clinical nor experimental support, in that no one substance was of itself toxic at the blood levels found in uraemia. They suggested that the symptoms seen were the result of the 'summated expression of a number of interplaying factors each of which in the last analysis is the result of the underlying disorder of function – failure to excrete the end products of metabolism'. In support of this view is the work of Olsen and Bassett (1951) who studied the blood levels of phenol, urea nitrogen, guanidine, and creatinine in uraemic patients and attempted to correlate them with the severity of the uraemia. They reported that the blood levels of phenol, although increased, 'could not be correlated with central nervous system depression nor could those of guanidine be correlated either with gastrointestinal stimulation or degree of elevation of blood pressure'. They concluded that 'uraemia remains a clinical entity without a direct known chemical basis'.

The organic substances known or reported to accumulate in uraemic blood include urea, creatinine, creatine, uric acid, certain amino acids, polypeptides, indican, hippuric acid, conjugates of phenol, phenolic and indolic acids and their conjugates, organic acids of the tri-carboxylic acid cycle, guanidine bases, acetoin and 2:3 butylene glycol (Simenhoff, Asatoor, Milne, and Zilva, 1963;

Kramer, Seligson, Baltrush, and Seligson, 1965). Simenhoff and his coworkers from a consideration of the methods used in the reported studies noted that the reported excess of polypeptides and guanidine bases was less certain than that of the other substances listed. That these substances accumulate in tissues and body fluids other than blood has been shown by many workers, including Simenhoff *et al* (1963), who measured aliphatic amines, and more recently Müting (1965), who measured glucuronic acid and free and bound phenols. These workers have shown that the levels of these retained products are higher than normal in the brain and other body fluids including the cerebrospinal fluid. It would now seem probable that some of these substances diffuse into the brain and other tissues and that the toxaemia of chronic renal failure is due to a summation effect of these organic compounds, possibly including urea, acting as enzyme inhibitors.

The possibility that some of the retained organic substances act as enzyme inhibitors has been investigated by various workers in an attempt to elucidate their role in the toxaemia of chronic renal failure. Giordano, Bloom, and Merrill (1962) studied the effect of urea on monamine oxidase at the concentrations found clinically in chronic renal failure. They reported that as the urea concentration in their assay system was increased enzyme activity decreased until a point was reached when maximum inhibition occurred. At higher urea concentrations there was less enzyme inhibition. Using 0·05 M urea solution, which is equivalent to a blood urea nitrogen level of 140 mg/100 ml, there was a significant degree of enzyme inhibition. Because of the action of monoamine oxidase in the destruction of serotonin and catecholamines, and its role in the regulation of amine metabolism in nervous tissue, inhibition of this enzyme could play a role in the pathogenesis of the uraemic syndrome. Hicks, Young, and Wootton (1964) studied the effect of phenolic acids on cerebral metabolism as measured by the rate of respiration and anaerobic glycolysis of guinea-pig brain slices and the inhibition of the activity of some selected enzymes involved in cerebral metabolism. The enzymes they studied were the decarboxylases of 3,4-dihydroxyphenylalanine, 5-hydroxytryptophan and glutamic acid, glutamic oxaloacetic transaminase, 5′-nucleotidase, amine oxidase, and lactic dehydrogenase. They showed that many aromatic acids, especially those with an unsaturated side chain, depressed the enzyme reaction rates. The concentration of phenolic acids used in their study was, however, higher than the reported levels for free aromatic hydroxy acids in uraemic plasma (Schmidt, McElvain, and Bowen, 1950). Hicks and her colleagues suggested that the lower blood concentrations of phenolic acids present in uraemic patients might possibly exert an effect by virtue of being present for a longer time than the relatively high concentrations in the acute experiments. It is also possible that the retained aromatic compounds could exert an enzyme inhibitory action by a summation effect *in vivo*. Young and Wootton (1964) also showed enzyme inhibition by aromatic and aliphatic amines, but compared with the phenolic acids, amines were less effective inhibitors of glutamic acid and di-hydroxy-phenylalanine decarboxylases. However, amines have been shown to cross the blood-brain barrier more readily than acids (Schwerin, Bessman, and Waelsch, 1950) and may therefore be more effective *in vivo* than *in vitro*. Recently Morgan and Morgan (1966) have reported elevated plasma levels of aromatic amines in uraemia and noted that the levels corresponded roughly with the elevation in the blood urea nitrogen levels. They also reported, as have others previously, that the levels fell markedly after dialysis.

That retained substances which are removed by dialysis are responsible for enzyme inhibition was shown in the experiments of Renner and Heintz (1965). They investigated respiration and metabolism of rat kidney and brain tissue slices using the sera of chronic uraemic patients as the incubation media. Under these conditions the tissue slices utilized less pyruvate, β-hydroxybutyrate, and acetoacetate than slices incubated in normal sera and the rate of formation of glucose from pyruvate was reduced. When serum from blood collected from the patients after dialysis was used, no inhibition was seen. They suggested that these differences in metabolism were possibly due to a reduction in adenosine triphosphate (ATP) synthesis in the presence of uraemic serum, and consequently a deterioration in the energy supply of the cell. That dialyzable toxic substances accumulate in the blood of patients with chronic renal failure was also reported by Henkin, Levine, Sussman, and Maxwell (1964) who studied HeLa cell growth *in vitro*. They reported that a toxic substance or substances to HeLa cells was present in the blood of 17 of 22 patients with chronic glomerulonephritis. After either haemo- or peritoneal dialysis the substances had been removed from the blood and there was evidence of cytotoxic properties in the dialysis fluid. The cytotoxic changes could not be induced with urea, creatinine, phosphate, uric acid, or potassium at the concentrations found clinically in renal failure. There was also no correlation between the degree of cytotoxic change induced and the level of blood urea nitrogen. In the serum of four patients with chronic pyelonephritis and in three of four patients with polycystic kidneys they were unable

to demonstrate cytotoxic effects. The significance of their findings is debatable, as cytotoxic material was also demonstrated in the serum of some patients with systemic lupus erythematosis, thrombohaemolytic thrombocytopenic purpura, and acute viral infections. Measurements of retained aromatic and aliphatic compounds other than urea, creatinine, and uric acid are not routinely made. Uric acid retention does occur and may occasionally give rise clinically to a 'gouty arthritis'.

ACIDOSIS

Although the retention of protein metabolites in chronic renal failure would appear to have far-reaching consequences, of equal importance to cellular metabolism are disturbances of acid base by their effects on the internal environment. The processes of intermediary metabolism give rise to a number of non-volatile acids and the hydrogen ions released from these acids must be excreted by the kidney if acid-base equilibrium is to be maintained. A normal individual on a normal mixed diet excretes an average of 60 m-equiv of total hydrogen-ion per day, of which 30 m-equiv is in combination with ammonia and 30 m-equiv as titratable acid. In chronic renal disease the production of acid is within normal limits, or slightly reduced if the patient is on a low-protein diet. The diseased kidney, however, excretes a lesser proportion of acid combined with ammonia and this proportional reduction, together with a reduced efficiency of titratable acid excretion, ultimately accounts for the acidosis of chronic renal failure. The reduction in acid excretion results from reduced functional renal mass, but there is evidence (Dorhout-Mees, Machado, Slatopolsky, Klahr, and Bricker, 1966) that ammonia excretion per nephron increases adaptively in the residual nephrons.

One of the remarkable features of the acidosis of chronic renal failure is that once it has developed it may remain remarkably stable for long periods of time and this is reflected in the steady low plasma bicarbonate levels of these patients. The mechanisms of this stability are of considerable interest and appear to be linked to another feature of the biochemical disturbances of chronic renal failure. Goodman, Lemann, Lennon, and Relman (1965) reported that, despite stable plasma bicarbonate levels, patients with severe chronic renal acidosis excrete significantly less acid in their urine than is produced by metabolic processes. They reported evidence which suggests that in chronic renal failure the acidosis evokes an extrarenal homeostatic mechanism that disposes of the endogenous acid not excreted in the urine. Lemann, Litzow, and Lennon (1966) have shown that in normal subjects with chronic metabolic acidosis induced by ammonium chloride loading this extrarenal mechanism is operative. They suggest that this extrarenal mechanism involves bone and that the acidosis may cause dissolution of bone by a direct effect of *p*H on the solubility of bone mineral, with other factors such as vitamin D resistance and secondary hyperparathyroidism playing a role.

The concept that bone plays a separate role in body buffering mechanisms is not a new one. It was originally proposed by Albright, Burnett, Parson, Reifenstein, and Roos (1946) who regarded the dissolution of bone salts as an important compensatory mechanism in the control of acid-base disturbances in renal disease. They demonstrated a marked increase in urinary calcium excretion in one patient of their own and another from the literature in whom chronic acidosis was induced by ammonium chloride loading. In their concept patients with either 'renal' acidosis or 'renal rickets' resulting from 'tubular-insufficiency-without' or 'with-glomerular-insufficiency', respectively, were in a state of negative calcium balance as the result of bone dissolution and excessive urinary calcium loss. They calculated that if the calcium losses were derived from the bone salts in the same proportions as they normally existed in bone, 0·49 m-equiv of acid would have been neutralized for each millequivalent of calcium lost. Reidenberg, Haag, Channick, Shuman, and Wilson (1966), in a study of metabolic acidosis induced by fasting in obese women, reported an increased urinary calcium excretion during the acidosis, and that the calcium loss was reduced by alkali therapy despite continued fasting. During the untreated acidotic phase the net negative calcium balance was 156 mg/day. They calculated that the buffer anions accompanying this calcium loss would combine with 4 to 8 m-moles H^+/day and act in controlling the extracellular acidosis. Raaflaub (1961) calculated that a 30% demineralization of the skeleton would liberate between 7,000 and 8,000 m-equiv of buffer base and proposed that the bones provide an almost inexhaustible base reserve for the animal organism. In his concept he went so far as to suggest that metabolic acidosis had, possibly, a direct stimulatory action on the parathyroid glands; as yet there is no evidence for such a controversial hypothesis.

Albright and his colleagues (1946) proposed that alkali therapy, by correcting the acidosis, would prevent the calcium loss in renal acidosis. Since then other workers have reported studies in which correction of the acidosis with alkali therapy in chronic renal failure has had no effect on bone demineralization. Stanbury, Lumb, and Nicholson (1960)

reported a case of chronic renal failure where the administration of alkali alone had no effect on the external mineral balances, although apparently correcting the acidosis. In three of the patients studied by Dent, Harper, and Philpot (1961) there was no change in calcium balance during treatment with alkali alone, despite correction of their acidotic state.

CALCIUM, PHOSPHATE, AND BONE

Disturbances in calcium and phosphate metabolism with associated metabolic bone disease are well accepted biochemical features of chronic renal failure. The disturbance in calcium and phosphate homeostasis commonly takes the form of hypocalcaemia and hyperphosphataemia, but these changes are not invariable. The metabolic bone disease associated with azotaemic renal failure has been described collectively by the terms 'renal osteodystrophy' (Liu and Chu, 1943) or 'renal-glomerular-osteodystrophy' (Dent *et al*, 1961) and consists of osteomalacia, osteitis fibrosa, and osteosclerosis. Bone disease in patients whose lives are being maintained by intermittent dialysis is one of the increasing clinical problems of this group. In chronic renal failure the plasma inorganic phosphate level rises; this is derived from the diet and may also be liberated from organic compounds of the tissues such as phospholipids and nucleoproteins in catabolic states. The increase in the plasma level could be due simply to a reduction of functional renal mass. However, although phosphate clearance decreases with advancing chronic renal disease, the rate of clearance falls proportionately less than that of glomerular filtration, so that the average rate of phosphate excretion per residual nephron increases as the nephron population is reduced. There is evidence (Slatopolsky, Gradowska, Kashemsant, Keltner, Manley, and Bricker, 1966) that in this mechanism parathyroid hormone is the important factor rather than the increase in glomerular filtration per nephron. The high phosphate level in itself is not known to produce skeletal disorders, but, in the classical concept of bone disease in chronic renal failure, phosphate is considered to have a secondary effect in lowering the plasma calcium concentration. It is well accepted that oral or intravenous administration of phosphate salts lowers the plasma calcium concentration in both normal and hypercalcaemic subjects. The mechanism of this effect has until recently been in some doubt, but the recent experiments reported by Hebert, Lemann, Petersen, and Lennon (1966) support the view that phosphate infusion lowers serum calcium concentration as the result of the precipitation in the body of $CaHPO_4$, by exceeding the solubility product of that salt.

In the classical concept, if changes in the plasma calcium concentration follow or are secondary to those in the plasma phosphate level one would expect plasma calcium levels to be low in all patients with chronic renal failure. Such is, however, not the case. The plasma calcium concentration may remain normal despite considerable increases in the plasma phosphate level; alternatively it may be low in the presence of only small changes in the phosphate concentration. An explanation for these findings has been proposed recently by Stanbury and Lumb (1966) who consider that the plasma calcium level in chronic renal failure is determined by factors other than the plasma phosphate concentration. Their view is based on their own personal experiences of a large series of cases of azotaemic osteodystrophy and they consider that in these patients there is a continuous spectrum of changes in which the cases with osteomalacia or defective mineralization and those with pure osteitis fibrosa or excessive decalcification represent the two extremes. They divided their patients into two groups. In group one, those with evidence of osteomalacia or defective mineralization, the plasma calcium was statistically subnormal. In their second group, the patients had no evidence of defective mineralization but had evidence of osteitis fibrosa, and in these the plasma calcium levels were normal. If these changes are not caused by alterations in the plasma phosphate other biochemical factors must be considered.

In those patients with defective mineralization a deficiency of calcium is present. It is well established that in chronic renal failure there is a reduced calcium absorption from the gut (Lichtwitz, de Sèze, Parlier, Hioco, and Bordier, 1960; Kaye and Silverman, 1965). Of the various factors affecting calcium absorption alterations in vitamin D sensitivity may be important, although recently Clarkson, McDonald, and De Wardener (1966) have reported evidence indicating that the impairment of absorption is secondary to the diminished urinary calcium excretion caused by the renal disease. There is, however, a considerable amount of evidence that in chronic renal disease there is an acquired insensitivity to vitamin D, as it has been shown that vitamin D produces a dramatic therapeutic response in those cases with defective mineralization only if given in very large doses (Stanbury, 1960; Dent *et al*, 1961). Recently Evanson (1966) has reported evidence compatible with vitamin D insensitivity, in that patients with hypocalcaemia in renal failure showed a diminished response to the calcaemic action of exogenous parathyroid hormone, a result similar to that seen after hormone administration in simple

vitamin D deficiency. It has been reported that the presence of vitamin D is necessary for the peripheral calcaemic effect of parathyroid hormone on bone, but not its renal effect (Rasmussen, Deluca, Arnaud, Hawker, and Von Stedingk, 1963; Arnaud, Rasmussen, and Anast, 1966); the converse, however, is not true. It is suggested by these authors that vitamin D and parathyroid hormone act synergistically at mitochondrial level. Other workers (Ney, Au, Kelly, Radde, and Bartter, 1965) have challenged these conclusions and from their own observations concluded that all the effects of parathyroid hormone are exerted in the vitamin-D-deficient state. These latter workers used experimental animals of a different species to those of Rasmussen and his colleagues, and it is possible that their results reflect species variation.

In those cases with excessive demineralization of bone and florid osteitis fibrosa the bone lesions are usually associated with marked parathyroid hyperplasia. The histological lesions in the bone in these patients are indistinguishable from those of primary hyperparathyroidism, suggesting that in this group the major factor is an excessive secretion of parathyroid hormone. That the lesions are due to hormonal excess is supported by the fact that subtotal parathyroidectomy with, presumably, removal of excess hormone production, produces clinical improvement in the bone lesions (Stanbury *et al*, 1960; Felts, Whitley, Anderson, Carpenter, and Bradshaw, 1965).

Some patients with chronic renal failure also show osteosclerosis (Kaye, Pritchard, Halpenny, and Light, 1960). These lesions are seen histologically as an excess of bone or hyperostosis rather than the production of abnormally dense bone. Kaye and Silverman (1965) regarded osteosclerosis as the most common bone lesion seen in renal failure and reported that it may be present simultaneously with either osteomalacia or osteitis fibrosa. They believe from their studies of bone formation rates in patients with chronic renal failure that the pathogenesis of osteosclerosis is a diminution in the removal of formed bone. Disturbances of calcium metabolism in chronic renal failure are obviously multifactorial and require considerable further elucidation.

MAGNESIUM

Although many studies have been reported on disturbances of calcium metabolism in chronic renal failure there are fewer reports on disturbances of magnesium metabolism. Magnesium is one of the major mineral constituents of the animal organism and its presence is essential for life. Magnesium activates some of the enzymes which split and transfer phosphate groups, among them the phosphatases and enzymes concerned in reactions involving ATP. Since ATP is required in such diverse functions as muscle contraction, the synthesis of protein, nucleic acid, fat and coenzymes, glucose utilization and oxidative phosphorylation, the activating action of magnesium, by inference, extends to all these functions. Hypermagnesaemia as a feature of chronic renal failure was first reported in 1923 (Salvesen and Linder, 1923) and has been reported by many others since then. Merrill (1965) stated that 'hypermagnesemia is constantly present in chronic renal failure'. Breen and Marshall (1966) reported raised plasma magnesium levels in predialysis specimens of uraemic patients with a significant fall after dialysis, although the levels were still higher than normal. In view of the role of magnesium in cellular metabolism it is not surprising that the hypermagnesaemia has been implicated as a cause of some of the neurological manifestations seen in patients with chronic renal failure (Hamburger, 1957; Randall, Cohen, Spray, and Rosemeisl, 1964). In contrast to this, however, is the work of Clarkson, McDonald, De Wardener, and Warren (1965) who reported normal levels of magnesium in 21 patients with chronic renal failure. It was also shown that magnesium balance in eight of the patients they studied was within normal limits. The absorption and urinary excretion of magnesium was less than in normal subjects, but there was some evidence to suggest that this was due to a diminished intake of magnesium. A link between calcium and magnesium disturbances in chronic renal failure is reported in the recent investigation of Clarkson, Warren, McDonald, and De Wardener (1967) who studied the effects of a high calcium intake either as citrate or carbonate on magnesium metabolism in both normal and uraemic subjects. In both groups there was a diminution in magnesium absorption and equivalent fall in urinary excretion, associated with a fall in plasma magnesium concentration. They suggested that the fall in plasma magnesium was due to an inhibition of parathyroid hormone secretion secondary to the rise in calcium absorption and plasma calcium concentration.

The factors affecting magnesium homeostasis and the role of parathyroid hormone are not clearly defined but it is accepted that in many clinical situations calcium and magnesium homeostasis are linked. Bulger and Gausmann (1933) concluded from experimental animal and human studies with parathormone that there was little direct evidence to suggest that the parathyroid glands had a direct effect on magnesium homeostasis. They noted that in the hyperparathyroid state, either induced with parathormone or due to an adenoma, there was a

negative magnesium balance. A relationship between urinary calcium and magnesium excretion was first shown in 1909 by Mendel and Benedict who reported that the infusion of calcium salts into dogs was followed by an increased urinary magnesium excretion. This observation has since been confirmed by others in both animals and man. A common distal tubular transport mechanism for these two ions was postulated by Samiy, Brown, Globus, Kessler, and Thompson (1960) from their experimental studies in dogs, using stop-flow techniques. Support for such a hypothesis is derived from the experimental studies of others (Ardill, Halliday, Morrison, Mulholland, and Womersley, 1962; Alcock and MacIntyre, 1962). Hanna, North, MacIntyre, and Fraser (1961) studied patients with primary hyperparathyroidism and attributed the negative magnesium balance to an increased urinary loss, as the result of an increased renal filtered calcium load, the consequence of a common tubular transport system. They concluded that there was no evidence, from their studies, to justify a 'primary action of parathyroid hormone on magnesium metabolism'. Subsequently, however, MacIntyre, Boss, and Troughton (1963) suggested that the parathyroid glands exerted a homeostatic effect by variations in renal magnesium clearance. Heaton (1965) considered that the glands exerted homeostatic control in a manner similar to that for calcium, by both promoting magnesium mobilization from bone and by decreasing renal clearance. Magnesium is predominantly intracellular and, like the major intracellular cation potassium, is held within the cell by active metabolic mechanisms. Disturbances of intracellular metabolism may cause the release of both these cations into the extracellular fluid. The effect of chronic renal failure on the homeostatic mechanisms controlling the serum magnesium concentration and the result of cellular metabolic disturbances requires further elucidation.

POTASSIUM

In acute renal failure potassium intoxication is a potentially lethal factor, but in chronic renal disease hyperkalaemia rarely occurs except in the terminal stage. Potassium is released from the cells as the result of protein catabolism and of acidosis. Strauss and Raisz (1956) calculated that the catabolism of 75 g of muscle protein released 33 m-equiv of potassium from cell water. In acidosis with an increased hydrogen ion concentration in the extracellular fluid, intracellular potassium is exchanged for extracellular hydrogen ions at the expense of the intracellular potassium concentration. Scribner and Burnell (1956) reported that there was a change of 0·4 to 1·5 m-equiv/l in the serum potassium concentration for every 0·1 unit change in extracellular *p*H. These alterations in serum potassium concentration are solely dependent on extracellular *p*H changes (Simmons and Avedon, 1959). The infrequency of hyperkalaemia in patients with chronic renal failure is usually attributed to the ability of the diseased kidney to maintain a normal level of urinary potassium excretion by tubular secretion (Leaf and Camara, 1949; Berliner, Kennedy, and Hilton 1950). In patients with chronic renal failure it has been shown that potassium clearance exceeds inulin clearance, in the advanced stages of the disease, as the result of tubular secretion (Platt, 1950; Mudge, 1956). It was later shown that the reabsorption of filtered potassium was essentially complete and urinary potassium was derived from that secreted in the distal tubule (Davidson, Levinsky, and Berliner, 1958). There is, however, some recent evidence (Hayes and Robinson, 1965) that patients with chronic renal failure have an increased faecal loss of potassium, which is not associated with overt diarrhoea, steatorrhoea, or obvious alteration in intestinal transit time. Whether such an increase in faecal potassium is due to diminished bowel absorption or increased bowel secretion is at present debatable.

SODIUM AND WATER

The kidney is directly implicated in disorders of sodium and water balance in patients with chronic renal failure. It is well known that patients with chronic renal failure have an impaired ability to conserve sodium, which is commonly seen as a mild hyponatraemia rather than as profound salt wasting. The mechanism of the hyponatraemia seen in chronic renal failure was investigated by Coleman, Arias, Carter, Rector, and Seldin (1966), who showed that patients with chronic renal disease are incapable of achieving sodium balance during rigid sodium depletion. They concluded that the salt wastage seen in chronic renal disease is due to the inability of the surviving nephrons to lower sodium concentration of tubular fluid below a relatively high fixed value and that the cause of this apparent gradient limitation is the increased osmotic load per nephron. The increased osmotic load per nephron is probably also responsible for the tubules' impaired inability to concentrate urine and this is related to the diminished response of the tubules to antidiuretic hormone.

In the early stages of chronic renal failure the impaired ability to conserve sodium and the associated hyponatraemia may necessitate the administration of sodium to prevent depletion. In the late

or 'end-stage' oliguric phase of chronic renal failure the situation is reversed and a different pattern of sodium homeostasis is seen, which is manifested by severe, uncontrollable hypertension. Comty, Rottka, and Shaldon (1964), in a study of the control of hypertension in patients with 'end-stage' chronic renal failure treated by intermittent haemodialysis, suggested that expansion of total body water and increase in exchangeable sodium played a role in maintaining the hypertension. In their patients before dialysis treatment there was an excess of total body water, with a high normal, or high extracellular fluid volume and an excess of exchangeable sodium, in the absence of overt oedema. At the end of six months of intermittent haemodialysis treatment, and strict low sodium intake between dialyses, when control of blood pressure was satisfactory the patients had normal total body water, exchangeable sodium, and extracellular fluid volume. Blumberg, Nelp, Hegstrom, and Scribner (1967) have also reported studies in which satisfactory control of hypertension in 'end-stage' chronic renal failure was achieved when extracellular fluid volume and exchangeable sodium were reduced to normal or near normal levels by intermittent haemodialysis and sodium restriction between dialyses. The problems of sodium and water balance are linked with the clinical problem of the hypertension in chronic renal failure due to primary renal disease and will not be discussed further here, except to state that hypertension of chronic renal failure and its effects can be reversed by adequate dialysis.

HORMONES

Sodium and water balance is normally regulated in part by aldosterone and antidiuretic hormone (ADH). In non-oedematous patients with chronic renal failure, disturbances of aldosterone secretion do not seem to play a dominant role, as Cope and Pearson (1963), in a study of aldosterone secretion rates in patients with chronic renal failure, found no correlation with the blood urea level, the urinary sodium or potassium excretion, nor with the concentration of either sodium or potassium in the serum. There is, however, evidence that the tubules in patients with chronic renal failure show a diminished response to adequate circulating levels of ADH (De Wardener, 1962). This failure in ADH responsiveness appears to be due mainly to the osmotic diuresis that is occurring in the individual surviving nephrons. Milne (1963) stated that 'in most cases of chronic renal failure the tubules become unresponsive to aldosterone, but occasionally sensitivity is preserved causing a combination of the signs and symptoms of uraemia and of the nephrotic syndrome'. Patients with chronic renal failure do not lose excessive amounts of sodium in their urine when on a normal salt intake, but lose the normal reaction of the kidney to salt deficiency.

In the female fertility is depressed and secondary ammenorrhoea or menorrhagia occur in chronic renal failure (Shaldon, 1966). In the male, fertility is reduced to a lesser extent than in the female and normal sexual activity with procreation has been reported (Shaldon, Baillod, Comty, Oakley, and Sevitt, 1964).

With regard to steroid excretion there is in chronic renal failure a reduction in the rate of cortisol clearance from the plasma after an intravenous infusion (Englert, Brown, Willardson, Wallach, and Simons, 1958). Englert and his coworkers reported that in their patients there was an inverse relationship between endogenous creatinine clearance and endogenous conjugated plasma 17-hydroxycorticosteroid (17-OHCS) levels. After cortisol infusion the rate of clearance of free plasma 17-OHCS was reduced, while the levels of conjugated 17-OHCS rose further and their clearance also was reduced. The defect in the clearance of the conjugated fraction was correlated with the degree of functional renal impairment. There was no correlation between the rate of free 17-OHCS clearance and the degree of renal insufficiency. They postulated that 'the accumulation of conjugated 17-OHCS in the plasma may have decreased the rate of removal of the free 17-OHCS by mass action'. In one patient with congestive cardiac failure and renal failure where the circadian variation in plasma 11-hydroxycorticoid concentration was measured, there was no change in the rhythm attributable to the renal failure (Connolly and Wills, 1967).

GLUCOSE

It is well known that patients with chronic renal failure have a reduced ability to handle a glucose load and that the degree of glucose intolerance correlates roughly with the severity of the uraemia. Many theories have been postulated to account for the abnormal carbohydrate tolerance in chronic renal disease and include defects in insulin synthesis or release, the presence of circulating insulin antagonists, and defects in peripheral glucose utilization or hepatic glycogen storage. Of these the current evidence appears to be in favour of the accumulation, in uraemic patients, of a circulating insulin antagonist or antagonists. Hampers, Soeldner, Doak, and Merrill (1966) have shown that the abnormal glucose tolerance associated with chronic renal failure can be corrected with adequate haemodialysis. They speculated that the low molecular

weight substances which accumulated manifested their effects by decreased insulin response to glucose loads and a peripheral insensitivity to insulin. Briggs, Buchanan, Luke, and McKiddie (1967) measured blood sugar and plasma insulin levels following an oral glucose load in uraemic patients. In these patients the blood sugar rose normally but remained significantly higher than in normal controls at 60, 90, and 120 minutes after the load. The plasma insulin levels, in these patients, showed a high fasting level with a normal rate of increase and a delayed fall when compared with controls. They considered that their results excluded a defect in either the synthesis or release of insulin as a cause of the glucose intolerance in uraemia and proposed that the defect in glucose metabolism in this condition was due to the accumulation of dialysable insulin antagonists.

The nature of such an antagonist or antagonists to endogenous insulin is, however, ill defined. An insulin response pattern similar to that seen in uraemic patients has been reported by Buchanan and McKiddie (1967) in maturity-onset diabetics. In this latter group of patients the impairment of glucose tolerance and insulin sensitivity is associated with raised plasma levels of non-esterified fatty acids (Nefa) (Randle, Garland, Newsholme, and Hales, 1965). In these patients the hypoglycaemic effect of insulin is frequently delayed until the plasma Nefa level has fallen (Hales and Randle, 1963). In uraemic patients, however, Hampers and his colleagues (1966) reported that there was no significant difference in the fasting plasma Nefa levels before and after the correction by dialysis of their glucose intolerance. It has been suggested that urea may of itself play an important role in the glucose intolerance of uraemic patients by directly interfering with carbohydrate metabolism (Perkoff, Thomas, Newton, Sellman, and Tyler, 1958). The report of Hampers *et al* (1966), who dialysed two uraemic patients with high bath urea concentrations and noted that they did not develop abnormal glucose tolerance, suggests that urea alone cannot be incriminated.

PROTEINS

Proteinuria is a characteristic feature of renal insufficiency and is present throughout most of the course of the renal disease. Various mechanisms have been postulated to account for the proteinuria and include increased glomerular filtration of protein, decreased reabsorption of the filtered fraction, and protein loss into the urine from the tubular cells. Of these the evidence appears to be in favour of an increased glomerular filtration of protein as the result of increased permeability of the glomerular membrane. Such a mechanism does not, however, account entirely for the serum protein changes seen in chronic renal failure. Ludewig and Chanutin (1938) studied the effects of renal insufficiency, induced by partial nephrectomy, in rats. They reported that the severity of the proteinuria increased with time and that to some extent dietary protein intake influenced the urinary protein excretion. Their experimental animals did not develop hypoproteinaemia despite the marked urinary protein loss. They concluded that their experimental evidence supported the earlier proposal of Bloomfield (1933) that the urinary protein loss in chronic renal disease in man did not entirely explain the hypoproteinaemia seen in these patients.

The hypoproteinaemia seen in uraemic patients may also be accounted for in part by the low protein diet given to control the blood urea concentration in these patients. There is, however, also evidence that in chronic renal failure there is a disturbance of protein metabolism. Herndon, Freeman, and Cleveland (1958), using balance studies in chronic renal failure, reported that the patients had increased endogenous nitrogen metabolism and required a higher protein intake than did a normal subject to remain in nitrogen balance. The findings of Herndon *et al* were consistent with either a reduction in nitrogen utilization for protein synthesis or accelerated protein breakdown. Lacy (1965) reported that isolated livers from uraemic rats showed increased amino-acid uptake and urea production, when compared with those from normal controls, on perfusion with the same load of exogenous amino acid. McCormick, Shear, and Barry (1966) studied the incorporation of radioactive labelled L-leucine into protein using an *in-vitro* technique with cell-free preparations of liver from uraemic rats. They reported evidence that the rate of hepatic protein synthesis, as assessed by this technique, was increased in uraemia, consistent with the earlier findings of Lacy (1965). They concluded that if their findings were related to the negative nitrogen balance seen in uraemic patients either the rate of protein degradation was increased or the uraemic liver synthesized an incomplete or abnormal protein. The hypoproteinaemia of chronic renal failure appears therefore to be accounted for in part by urinary loss, in part by low dietary intake, and in part by some defect in protein metabolism, the nature of which is not at present well defined.

The nature of the alterations in serum protein fractions in patients with chronic renal failure are of interest. In a large group of patients with chronic renal failure both untreated and on long-term intermittent haemodialysis therapy, the levels of total serum protein and albumin fraction ranged

from high to low, while the values of the globulin fraction were either normal or high (Moorhead and Wills, unpublished data). In the early stages of chronic renal failure the globulin fraction concentration tends to be low but the levels increase with advancing renal failure. This alteration in the globulin fraction may be associated with the fact that all uraemic patients show an abnormal immune response. Hume, Merrill, Miller, and Thorn (1955) noted that after renal homotransplantation in chronic renal failure there was prolonged survival of the transplant with a much less intense local immune response than was expected from transplantation experiments on animals. Dammin, Couch, and Murray (1957) studied the survival of skin homografts in patients with chronic renal failure and reported prolonged graft survival. They suggested that if the rejection of homografted tissue were an immune response on the part of the recipient, then the delay seen in uraemic patients represented an impairment of this immune response. Similar results in experimental animals were reported by Smiddy, Burwell, and Parsons (1961). A decrease in both immediate and delayed cutaneous hypersensitivity reactions in patients with uraemia was reported by Kirkpatrick, Wilson, and Talmage (1964). They also reported that after a renal transplant the recipient acquired delayed responsiveness with a specificity identical with that of the kidney donor. These observations could not be explained either as alterations in the recipient's nutritional status or due to an improvement in the uraemic syndrome. They concluded that this passive transfer indicated that the cause of the depressed cutaneous hypersensitivity in uraemia was not due to 'an inability of the skin *per se* to react'. Rowlands, Wilson, and Kirkpatrick (1964), from histological studies, concluded that the alteration in cutaneous sensitivity was probably the result of an abnormality in the production of specifically sensitized cells. The factor or factors affecting the immune response in patients with chronic renal failure and the relationship with protein metabolism still require further elucidation.

IRON AND HAEMOGLOBIN

Alterations in iron and haemoglobin metabolism are seen in patients with chronic renal failure and contribute to the problem of anaemia in this group. The anaemia of chronic renal failure is classically both normochromic and normocytic and it seems probable that this is a reflection of an alteration in cellular metabolism as the direct result of the retention of 'organic compounds', although the anaemia is not corrected by adequate dialysis. In patients with chronic renal failure evidence of bone marrow depression has been reported, as demonstrated by a diminution in the rate and amount of radioactive iron utilized for haemoglobin synthesis (Joske, McAlister, and Prankerd, 1956; Loge, Lange, and Moore, 1958). The defect in iron utilization is usually attributed to an inadequate production of erythropoietin. The existence of the role of this humoral factor which mediates the stimulus to erythropoiesis was first reported by Reissmann in 1950, and its renal origin was demonstrated in experimental animals by Jacobson, Goldwasser, Fried, and Plzak (1957) and by Goldwasser, Fried, and Jacobson (1958). These authors reported that after bilateral nephrectomy experimental animals failed to show an elevation of plasma erythropoietin concentration following either cobalt chloride, or acute haemorrhage, or reduced barometric pressure. Further evidence that erythropoietin was produced in the kidney was reported by Naets (1960) who later reported that erythropoietin production was impaired in patients with chronic renal insufficiency (Naets and Heuse, 1962). It had been reported earlier that erythropoietin was absent from the plasma of uraemic patients with anaemia (Gallagher, McCarthy, Hart, and Lange, 1959). That the kidney of itself plays a dominant role in the anaemia of chronic renal failure was demonstrated in experimental animals by Suki and Grollman (1960). These authors reported a marked reduction in erythropoiesis and haemoglobin synthesis in nephrectomized dogs despite control of azotaemia with intermittent peritoneal dialysis. The dominant role in the aetiological mechanisms of the anaemia of chronic renal failure appears to be deficient erythropoiesis consequent upon a diminution in erythropoietin production, with other factors playing a contributory role.

Alterations in iron metabolism and transport in renal failure are a complex problem, and Brown (1964), in a study of plasma iron transport rates during haemodialysis, could show no correlation between the direction of the change in the transport rate, the percentage fall in blood urea, the change in red cell mass, or the plasma iron.

Other factors which contribute to the anaemia and which are also associated with the fundamental biochemical disturbance are haemolysis and the haemorrhagic diathesis which are seen in these patients. Joske *et al* (1956) reported an increased red cell destruction in uraemia and suggested that this was due to an extracellular factor acting on normal red cells rather than to any cellular defect *per se*. The erythrocytes are themselves normal, as measured by their ability to survive normally in healthy recipients (Loge *et al*, 1958). In a recent study of red cell survival in patients with chronic renal failure Shaw (1967) reported that the red cell life span was

normal in 10 patients with blood urea levels of less than 135 mg/100 ml and was reduced in nine of 10 patients with blood urea levels of more than 200 mg/100 ml. He concluded that the reduced red cell survival in patients with chronic renal failure may be caused by the retention of the products of protein metabolism which accumulate in plasma approximately parallel to the blood urea level. Another aspect of the haematological disturbances seen in chronic renal failure, which is also associated with the biochemical disturbance, is the haemorrhagic diathesis. Castaldi, Rozenberg, and Stewart (1966) and Stewart and Castaldi (1967) have published evidence that uraemic bleeding is due to a qualitative defect of platelets, and that the defect was improved by dialysis, suggesting that retained low molecular weight organic compounds play an important role.

ENZYMES

In acute renal failure raised serum enzyme levels have been reported. Kemp, Lange, Laursen, and Nielsen (1964) reported increased serum levels of lactate dehydrogenase and of aspartic and alanine transaminases in the early phase of the anuric episode. In chronic renal failure Ringoir, Wieme, and Regnier (1965) have reported an elevation in the total lactate dehydrogenase (LD) activity and also noted an increase in the isoenzyme LD 5 fraction in post-dialysis blood specimens in acute or chronic tubular failure, whereas in patients with chronic pyelonephritis there was no difference in the pre- and post-dialysis specimens. The significance of this increase in LD 5, which is not the isoenzyme of renal origin, after dialysis in tubular disease is at present not clear.

VITAMINS

Alterations in vitamin metabolism occur in chronic renal failure, and Matthews and Beckett (1962) found abnormally high levels of vitamin B_{12} in the serum of 14 out of 32 patients with chronic renal failure and considered that a failure of renal clearance of the vitamin was the most likely explanation of their findings. Lasker, Harvey, and Baker (1963) measured the serum levels of folic acid, vitamin B_{12}, thiamine, biotin, pantothenic acid, and nicotinic acid in patients with chronic renal failure. They found abnormally low levels of folic acid with values ranging from high to low for biotin and nicotinic acid which seemed to be related to the duration of the uraemic state and the nutritional history. The vitamin B_{12} levels differed from those reported by the previous workers in that the values were below normal in one and raised in one out of a total of five patients. In their patients Lasker *et al* found no consistent change in the blood vitamin levels before and after dialysis. In patients with chronic renal failure maintained on intermittent haemodialysis water-soluble vitamins are considered to be removed during dialysis and oral supplements are given, despite adequate dietary intake (Shaldon, 1966).

CHOLESTEROL AND LIPIDS

Lipaemia in patients with chronic renal failure was originally noted by Bright. In 1911 Chauffard, Laroche, and Grigaut reported high serum cholesterol in patients with chronic renal failure which fell with increasing nitrogen retention, and they suggested that there was an inverse relationship between urea and cholesterol. Similar findings were reported by Henes (1920) and Ashe and Bruger (1933); the latter considered that the fall was due to cachexia. Page, Kirk, and Van Slyke (1936) measured plasma cholesterol and total lipids in patients with terminal renal failure and reported that the levels fell to either normal values or below when the urea clearance fell to less than 20% of normal. Disturbances of cholesterol and lipid metabolism are classically seen in association with the nephrotic syndrome. In patients with chronic renal failure not associated with the nephrotic syndrome serum cholesterol levels are usually within the normal range, while total serum lipids show a marked increase (Moorhead and Wills, unpublished data). The cause of the hyperlipidaemia is not clear, but it is probably related to the high fat diet that the patients are given as part of their therapeutic regimen rather than to an alteration in fat metabolism.

CONCLUSION

Chronic renal failure involves a fundamental disturbance of the constitution of the internal environment which has far-reaching biochemical consequences. There are in conclusion two points worthy of stress. The first is that in chronic renal failure many of the changes might be regarded as simply the end result of a reduction in functional renal mass. Such is, however, not the case. Despite a considerable fall in nephron population the surviving nephron units undergo functional adaptative changes which tend to delay the onset of the terminal phase of chronic renal failure. The functional changes that occur in the surviving nephron in chronic renal disease are described in what has been called the 'intact nephron hypothesis' (Bricker, Morrin, and Kime, 1960; Bricker, Klahr, Lubowitz, and Rieselbach, 1965), which was originally proposed by Platt in 1952. In this hypothesis the individual remaining nephrons show an adaptive increase in glomerular filtration

rate in response to a decrease in the total nephron population. The increased glomerular filtration rate in the remaining functional nephrons modifies the normal glomerulotubular balance to allow more salts and water to enter the tubules than the reabsorptive mechanisms can handle. There are also increases or decreases in the tubular capacity for active secretion and reabsorption. These alterations in glomerular filtration and tubular handling combine to compensate for a reduction in the total number of functional nephrons by tipping the glomerulotubular balance in the direction of excretion.

The second point is that the changes in the internal environment seen in chronic renal failure are complex and multifactorial. A change in any one system is often reflected in many if not all of the others and the Figure attempts to show some of the changes and their related consequences. Chronic renal failure results in the retention of urea and other metabolic products or 'organic compounds'. These retained products probably have a direct effect on cellular metabolism acting perhaps as enzyme inhibitors. The retention of urea has a direct effect on sodium and water balance by promoting an osmotic diuresis. The retained metabolic products contribute directly to the changes in acid-base balance. The acidosis of chronic renal failure of itself directly affects cellular metabolism and potassium balance; it also, either directly or through alterations in parathyroid hormone or vitamin D sensitivity, affects calcium, phosphate, and magnesium balance. Whether or not the acidosis directly affects calcium balance is controversial, as are the effects of the retained 'organic compounds' on vitamin D and parathyroid

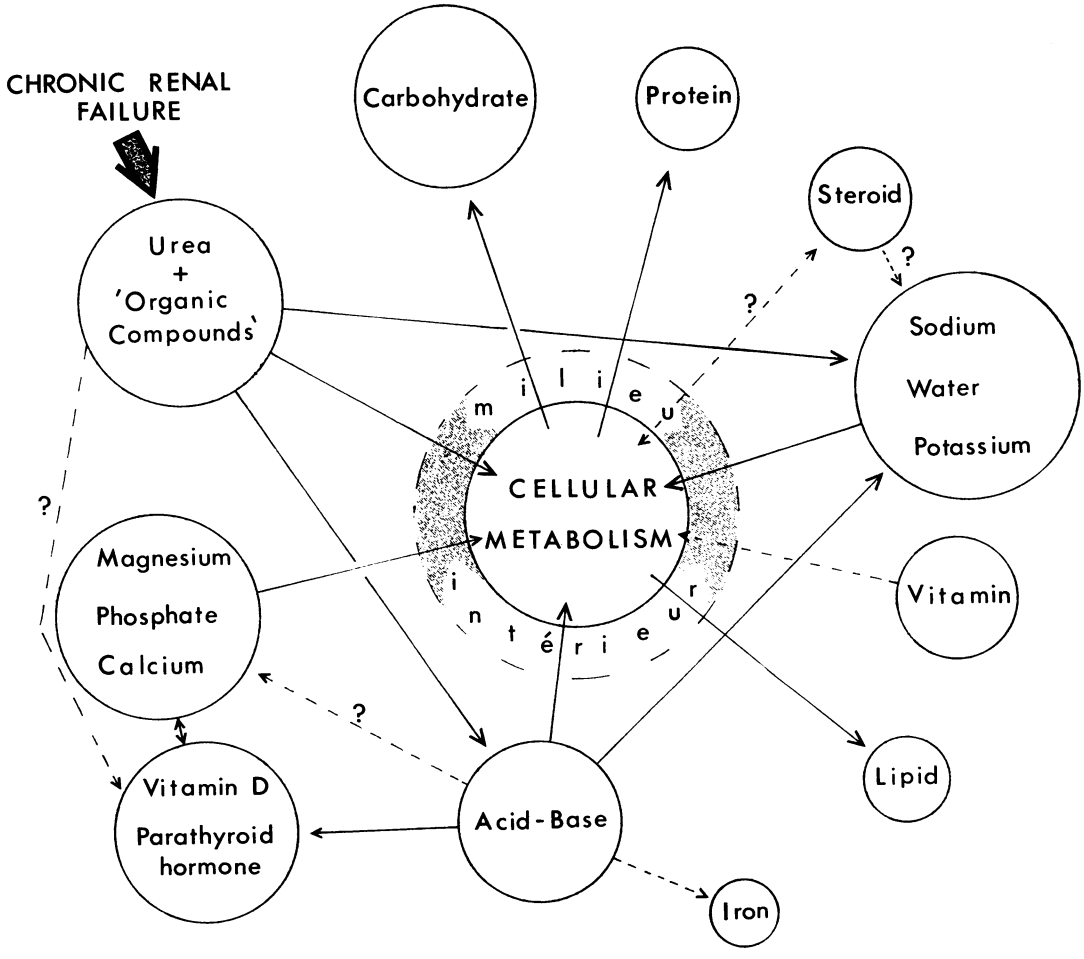

FIG. 1. *Diagram illustrating the changes in the internal environment in chronic renal failure.*

hormone sensitivity. The role of the elevation of the plasma phosphate concentration in the disturbances of calcium and magnesium balance is also controversial. The disturbances in calcium and magnesium balance of themselves contribute directly to the alterations in cellular metabolism. The alterations in cellular metabolism are reflected in disturbances of lipid, protein, carbohydrate, and possibly steroid metabolism: in the alterations in carbohydrate metabolism insulin antagonists probably play a dominant role.

The biochemical mechanisms involved in the uraemic syndrome of chronic renal failure are far from clear and it is a chastening thought that our knowledge of this subject has advanced very little in almost one hundred and forty years. In 1831 Richard Bright wrote in his observations on the deranged action of the kidney and its effects on cerebral function that 'how far the presence of urea, or the abundance, or deficiency, of any other principle in the blood, may be the immediate cause of such a degree of cerebral irritation as is capable of producing epilepsy, is a fair subject of enquiry'. It seems that, even today, the biochemical disturbances of chronic renal failure and their effects in producing the clinical syndrome of uraemia remain 'a fair subject of enquiry'.

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