METABOLIC ALTERATIONS CAUSED BY URAEMIA

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The knowledge of metabolic alterations in uraemia has reached an extent which is beyond the scope of this review. The mechanisms and classification of metabolic alterations will be considered here because of their impact on further research and treatment.

Metabolic alterations in uraemia could be classified into the following groups: accumulation, deficiency and regulatory imbalance.

Accumulation

This is the best known consequence of renal failure and dialysis has been developed primarily to correct this alteration. Various solutes accumulate in the uraemic subjects because of impaired elimination, increased synthesis or combination of both mechanisms.

Decreased excretion

There are numerous examples, such as water and electrolyte retention. However, some solutes excreted by the kidney are not primarily intended to be excreted. For instance amino acids or peptides are resorbed from the primary urine with an efficiency of over 90 per cent and only a small part of them is excreted [1, 2].

Non-excretory renal elimination

Kidney elimination is not restricted to excretion. For instance most peptides are taken up by the luminal as well as basolateral membranes of proximal tubular cells and broken down in the cells. Moreover, some peptides are split directly in the lumina of the proximal tubules by the peptidases present and the released amino acids are reabsorbed by the ordinary transport systems. These mechanisms are of the utmost importance for the biological half-life of various peptides, for instance peptide hormones [3]. By this mechanism the kidney participates in the humoral regulations of the organism.
*Extrarenal elimination*

Several mechanisms participate in this type of elimination. Phenylalanine (Phe) is an essential amino acid (EAA) of which the intake is low on a low protein diet. But it still accumulates because of inhibition of Phe hydroxylase by an inhibitor present in uraemic sera [4]. Non-essential amino acids (NEAA) accumulate in the ECF of uraemic subjects because of inhibition of protein synthesis as a result of EAA deficiency and the accumulation of an inhibitor of cell membrane amino acid transport [5].

*Increased synthesis*

There are various causes of increased synthesis.

(i) Increased catabolic rate, e.g. urea synthesis increases because of protein breakdown.

(ii) Alternative metabolic pathways: the inhibition of Phe hydroxylase causes decreased tyrosine synthesis and Phe is metabolised by an alternative metabolic pathway to phenylpropionic and phenylacetic acids and other metabolites.

(iii) New regulatory balance: the biological half-life of most peptide hormones is prolonged because of decreased elimination. The decrease in elimination should be counterbalanced by decreased synthesis because of feedback mechanisms. If not, other regulatory mechanisms participate in the increased blood concentration of these hormones. It is highly probable that this alteration is not restricted to hormones but also applies to other middle molecular substances.

*Deficiency*

Deficiency is in principle corrected by supplementation. Effective dialysis usually augments the signs of deficiency.

*Low intake*

Even a diet with normal caloric intake could be deficient in important nutrients such as EAA, vitamins, trace elements, etc.

*Increased loss*

The loss of solutes such as EAA, vitamins etc. during dialysis must be covered by increased intake. Vitamin and EAA supplementation schedules have been elaborated to assure the needs of the uraemic individual.

*Decreased synthesis*

Some examples: (i) lack of kidney parenchyma results in decreased 1,25(OH)₂ vitamin D₃ synthesis with the well described sequence of events [6].
(ii) Decreased synthesis of renal erythropoietic factor contributes to anaemia [7].
(iii) Decreased synthesis of histidine contributes to its deficiency [8]. (iv) The inhibition of Phe hydroxylase causes a decrease in blood tyrosine concentration.

Regulatory imbalance

A great deal of metabolic alterations in uraemia originate from the accumulation and deficiency changes. However, some alterations are not explained just by these changes and an additional mechanism, i.e. regulatory imbalance, has been suggested. This mechanism could be, in principle, described also in accumulation and deficiency changes, but it differs in pathogenesis.

Insufficient data on regulatory imbalance hinder classification such as is possible for accumulation and deficiency. Elucidation of the mechanisms still needs to be generally accepted. This is why a different approach will be used in the following part of the lecture.

Middle molecular substances (MMS)

The idea of MMS has been developed to explain the unsatisfactory clinical results of haemodialysis [9, 10]. The significance of MMS of 300–2500 mol wt has been stressed because of their dialysis through conventional dialysis membranes [11]. The idea of MMS appeared to be very fruitful.

A new methodology for the fractionation and determination of MMS has been developed. In principle there are two alternatives: either to use just gel filtration which measures several crude fractions in a large molecular range [12, 13], or to take one of these crude fractions and fractionate it further by means of ion exchange chromatography [14]. The second alternative enables us to measure a small part of better subfractionated MMS. However, even the subfractions are non-homogeneous. Unfortunately each scientific group modifying this methodology uses different fractionation conditions and nomenclature, which makes the whole literature hardly comparable. To enable at least a rough comparison a scheme of nomenclature has been constructed [15].

The square metre-hour hypothesis was formulated as the first of the hypotheses for predicting dialysis schedules [16]. Later on the dialysis index and other measures reviewed recently [17] were formulated.

The concept of MMS has influenced the development of dialysis membranes, machines, and though it respected the principle of accumulation as a primary cause of uraemic toxicity, the concept was an important impetus for the development of further ideas.

Trade-off hypothesis

The formulation of the trade-off hypothesis [18] enabled us to explain metabolic alterations in a new way. The cause of metabolic alterations need not be an unexcreted metabolic end product but a humoral regulator such as parathormone (PTH). The increase of PTH concentration is not a consequence of decreased elimination
but primarily the result of inappropriate synthesis caused by the low Ca concentration. The increased PTH concentration improves serum Ca concentration but reflects in significant side effects contributing to uraemic toxicity.

Similarly, serum immunoreactive insulin (IRI) concentration depends upon the glucose concentration in dialysate. If no glucose is present, serum glucose and IRI concentrations are low (Figure 1). Dialysate with 10mmol/L glucose does not influence glycaemia significantly because of the increase of IRI concentrations and resulting increase of glucose uptake by insulin sensitive tissues.

![Graph showing glucose concentration in dialysate](image)

Figure 1. The effect of glucose concentration in dialysate on glucose and IRI levels in serum measured after haemodialysis (Opatrný K, et al. Unpublished data)

What are the implications of these findings?

(i) Small molecules, i.e. Ca or glucose determine the levels of parathormone or insulin. These are good examples of the relationship between small (SMS) and MMS, and could explain good results without direct elimination of MMS by dialysis.
Figure 2. The fractionation of sera on a column of Sephadex G 15 (A) and the position of individual inhibitors (B) in fractions of MMS
(ii) Though the differences in the concentration of SMS are small, the changes of regulator concentrations are striking.

(iii) MMS of 300–2500 mol wt which are partly eliminated during dialysis are just a special case when both correction of SMS and the elimination of MMS by dialysis take part.

(iv) The suggestion that changes in the concentration of regulators participate in uraemic toxicity may have an outstanding impact in two ways. It has been postulated that parathormone is the principal uraemic toxin [19] responsible for various aspects of uraemic toxicity. On the other hand the search for various toxins and the search for uraemic inhibitors has increased strikingly.

Inhibitory activities

It would be difficult to present the complete list of inhibitory activities described in uraemic serum, urine or ultrafiltrate. A small part of them has been defined by a simple but effective gel filtration on Sephadex G 15 (Figure 2). Fraction 1, resembling those of a and b of Funck-Brentano or 8 and 9 of Bergström’s group, contain most of the described inhibitors; inhibitors of haemopoiesis (HEM) [20]. DNA synthesis [21], gluconeogenesis (IGN) [22], one of the natriuretic factors [23] and the neurotoxic substance [24]. It is probable that some of these are identical substances. Their mol wt appears to be over 1500 but it is not necessarily so.

Fraction 2, resembling seven of Bergström’s group or fraction c of Funck-Brentano’s group, contains the inhibitors of lymphoblastic transformation described by Bergström’s [25] and Hanicki’s [26] groups (probably identical substances) and the inhibitor of amino acid transport [5]. Both fraction 2 and 3 contain the inhibitors of haemoglobin synthesis [13] and lipoprotein lipase [27].

Additional inhibitors in fraction 3 are the inhibitor of glucose utilisation [28] and the inhibitor of lactate dehydrogenase [29]. Between fraction 4 and 5 an inhibitor of phagocytosis has been described [30].

Isolation

The first step in the search for these inhibitors is partial or complete purification. Various techniques can be used for the isolation procedure [22, 28, 31]. The selection of techniques depends mainly on the nature of the inhibitor.

Testing system

The crucial point for the isolation of an inhibitor is a sensitive testing system. With low sensitivity a large amount of the isolated material is needed. This is probably not important in the first steps of isolation, but it becomes important with further isolation steps. In vivo systems are usually of low sensitivity because the intact kidney of the experimental animal breaks down the inhibitor. In vitro systems include perfused organs, slices, intact cells, homogenates or subcellular structures such as mitochondria.
Structure definition

This is an important step for the definition of the kind of inhibitor, revision of the isolation procedure and eventual synthesis in the case of low molecular weight peptides.

The mode of action

The mode of action can be studied in principle even with unpurified inhibitors to obtain the first evidence concerning the likely value of a further search for the inhibitor. Unfortunately, many interfering solutes in complex systems such as serum or urine make sophisticated studies quite impossible. Therefore partial or complete purification has to precede studies on the mode of action of any inhibitor. The inhibitor of glucose utilisation was found to inhibit glucose utilisation in various tissues. The site of inhibition was found to be P-fructokinase and the type of inhibition was of a non-competitive nature [32].

The inhibitor of gluconeogenesis acts at the level of P-enolpyruvate carboxykinase [33]. It inhibits gluconeogenesis with a lag phase of about 30 min and

![Graph showing the effect of Ca concentration in Krebs Ringer bicarbonate on glucose production by rat kidney cortex slices and the interference of IGN with Ca stimulated gluconeogenesis.](image)

Figure 3. The effect of Ca concentration in Krebs Ringer bicarbonate on glucose production by rat kidney cortex slices and the interference of IGN with Ca stimulated gluconeogenesis. (For details of methodology cf [22])
Figure 4. The effect of adrenaline and insulin on gluconeogenesis by kidney cortex slices and the interference of IGN with their effect. Adrenaline (50μg/100g) and insulin (0.5U/100g) were applied to rats one hour before experiment subcutaneously and IGN was added to the Ringer bicarbonate containing 5mmol/L alpha-oxoglutarate. Incubation period: one hour at 37°C.

because of its glycopeptide structure and mol wt of about 17,000 [22] it has been suggested that it binds to the cell membrane and its effect is mediated by a second messenger. It was found that IGN completely inhibits Ca-stimulated gluconeogenesis (Figure 3). It retains inhibitory activity in the case of adrenaline-stimulated gluconeogenesis (Figure 4) but does not add to the inhibitory action of insulin.

Conclusions

A number of inhibitors participate in the metabolic alterations of uraemia and their significance varies. In the case of carbohydrate metabolism two inhibitors have been defined; an inhibitor of glucose utilisation and an inhibitor of gluconeogenesis, and it is still possible that additional inhibitors will be found. No doubt they interact
with known hormones such as insulin, adrenaline and glucagon. Though it is
dangerous to extrapolate from this kind of study, it is suggested that part of the
metabolic alterations of uraemic toxicity is caused by a chain of regulatory im-
balances. The study of regulatory alterations has increased significantly during
the last few years and there is a good chance that the results of these studies and
further increase in this knowledge of deficiency and accumulation changes, will
improve the treatment of uraemic patients in the near future.

References

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Open Discussion

BERGSTROM (Chairman) Thank you very much Professor Dzuirk for your very interesting and very clear presentation of this very complicated subject. I think you all realise that the whole problem of uraemic toxicity is very complicated and Professor Dzuirk has pointed out the difficulties which different groups have had to compare their results with regard to the separation procedures and so on. For instance this peak 7 which we used to isolate contains the neurotoxic peak of Professor Funck-Brentano. We exchanged our samples directly.

FRÖHLING (Potsdam) In co-operation with Professor Kokot we found a significant correlation between the middle molecule fraction 2 which corresponds to the peak 7 of Bergström and Fürst, and PTH plasma levels in patients with chronic renal failure. This correlation was proved in 46 patients with chronic renal insufficiency on conservative treatment including low protein diet. These findings suggest that PTH may be involved in the pathogenesis of uraemic toxicity mediated by middle molecules.

DZURIK I have nothing to add, I would like just to say that Professor Massry would be very grateful to see the correlation between parathormone concentration and the concentration of fraction 7. It depends on the method that is used.

BERGSTROM We made a similar observation. When we parathyroidectomised patients with high PTH and hyperparathyroidism we saw that the level of some of the middle molecule fractions decreased dramatically after parathyroidectomy, so we also think there is some sort of relationship between these two.

FUNCK-BRENTANO (Paris) We need to see how the methodology we use fits together and then speak about the same parameters getting some new correlations.

BERGSTROM You mentioned that you find your inhibitor of glucose utilisation in a fairly low molecular fraction in plasma, but you find it in a fairly high molecular fraction in the urine.

DZURIK It was so. In plasma and in the urine the inhibitor of glucose utilisation was present in fraction 3, but in the high molecular range there was the inhibitor of gluconeogenesis. These are two different substances, the one that is the inhibitor of glucose utilisation acting at the level of phosphogluco kinase, the second one which inhibits gluconeogenesis is acting at the level of phosphopyruvatecarboxykinase. These are completely different substances.

BERGSTROM I think this also suggests that we may have in the future to realise there are uraemic toxins which have much higher molecular weights than the so-called middle molecules. Of course, as you pointed out, the normal kidney eliminates also larger molecules by digestion in the tubules, so it is reasonable to think that if we have kidney failure with no kidney function available, we should have an accumulation of potentially toxic high molecular weight substance.

DZURIK I am sure you are right.
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NEPHROLOGY 1

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NEPHROLOGY 2

Chairmen: A Struyvenberg
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