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INVESTIGATING UREMIC SYNDROME THROUGH EXPERIMENTAL MODELS IN RATS

Discoveries Regarding Chemical Composition in Blood and Urine

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Abstract: A chronic uremic syndrome was induced in rats through a 5/6 nephrectomy, while an acute uremic syndrome was induced through bilateral nephrectomy. In the chronic uremic syndrome, analysis of serum and urine revealed significantly elevated levels of BUN and creatinine. Additionally, the animals exhibited chemical indicators characteristic of secondary hyperparathyroidism. They experienced severe polyuria, proteinuria, and albuminuria, although surprisingly, the total serum protein levels were increased. The experimental chronic uremic syndrome can be described as a syndrome of "extreme adaptation," affecting both the regulatory activity of the remaining renal parenchyma and the overall body. On the other hand, the acute uremic syndrome induced by bilateral nephrectomy is a severe disorder of water and electrolyte metabolism, ultimately leading to the death of the experimental animals within 50-60 hours.

Key words: Experimental uremic syndrome -- Rat -- 5/6 reduction of renal parenchyma Serum- and urine analysis -- Secondary hyperparathyroidism.

Introduction. Uremic syndrome is a condition that affects the entire body. It has been found that a general disturbance in cellular metabolism is the cause of functional impairments in various organs (Bergstrom & Bittar, 2009; Teschan, 2010). There is some evidence that the main impact is observed on oxidative phosphorylation in mitochondria (Morgan et al., 2003; Lennar, 2008; Heitz et al., 2011). However, the exact pathogenesis of this disorder is not yet fully understood. The following pathogenetic mechanisms have been proposed:

1.Imbalance of electrolytes, particularly calcium and magnesium ions (Kimich & Lassmussen, 2009).

2.Disturbance in acid-base balance leading to acidosis (Lellman, 2012).

3.Formation of "uremic toxins" (reviews: Teschan, 2010; Dobbelstein, 2011).

Renal insufficiency can be induced in rats by a sharp decrease in the total number of functioning nephrons, bilateral nephrectomy, or 5/6 reduction of renal parenchyma. These procedures are based on the following considerations: 1. The degree of loss of functioning renal parenchyma is the determining factor for renal dysfunction, regardless of the underlying disease (Bricker et al., 2015), and 2. in rats older than 2 months, there is no further increase in the number of nephrons (Bonvale et al., 2002). The present experiments were conducted to obtain a spectrum of functional parameters characterizing experimental uremic syndrome in rats. These parameters will be correlated with the results of stereological and biochemical analysis of liver tissue and data on calcium and strontium metabolism in rats with uremic syndrome (Remagen et al.). Thus, the aim of these experiments was to gather information on functional parameters characterizing uremic syndrome in rats. These data will be compared with the results of stereological

and biochemical analysis of liver tissue, as well as data on calcium and strontium metabolism in rats with uremic syndrome (Remagen et al.)

Materials and methods.

1.Chronic uremic syndrome. We used a modified technique described by Chanutin and Ferris (1982), Platt et al. (1952), and Morrison (2006). The left kidney of 44 male Wistar inbred rats (body weight 175-190 g, Hoffman-La Roche, Ltd, Basel) was subcutaneously implanted in the back using a lateral incision under ether anesthesia.

Reduction of the parenchymal volume to one-third of the original size was achieved by resecting both poles of the kidney. After 8-10 days, the entire right renal organ was removed. In the control group of 21 animals (body weight 175-195 g), unilateral nephrectomy was performed.

2.Acute uremic syndrome. It was induced by bilateral complete nephrectomy in 8 male Wistar rats (body weight 190-210 g). Six rats with sham surgery (body weight 190-210 g) and 3 untreated rats (body weight 200-210 g) were used as controls. All animals were housed in plastic cages with 2 rats per cage. They were given standard laboratory feed (Altromin-t˜) and had free access to drinking water from plastic bottles.

3.Serum analysis. The following measurements were performed on 12 animals with 5/6 nephrectomy, 21 animals in the control group, as well as on all experimental animals with bilateral nephrectomy and the control group: blood urea nitrogen (BUN), creatinine, phosphates, chlorides, albumin, total protein, alanine aminotransferase (SGPT), and alkaline phosphatase on an autoanalyzer; sodium, potassium, and total calcium by flame photometry (Eppendorf photometer). Blood samples were taken from the retroocular plexus on the 20th, 40th, and 84th day after the right kidney removal, as well as 48 hours after bilateral nephrectomy.

4.Urine analysis. Urine from 3 rats with 5/6 nephrectomy and 3 control animals was collected over a period of 3 days (from the 72nd to the 74th day after the right kidney removal). The 24-hour urine volume, osmolality, and pH were measured, in addition to the determinations performed in the serum. The animals were euthanized by decapitation on the 85th day after the right kidney removal and 48 hours after bilateral nephrectomy.

Results.

1.Chronic uremic syndrome

Serum analysis: A significant and sustained increase in the concentration of BUN and creatinine, the only values exceeding the normal range for the methods used, provides confirming evidence of the presence of chronic uremic syndrome. Statistically significant differences were found between experimental and control animals in the levels of calcium, potassium, and chloride. Total protein was elevated, while inorganic phosphorus and albumin were decreased. Compared to values determined on the 20th and 40th day after right kidney removal, there is a slight increase in BUN (20 days: 77.6, 40 days: 83.2 mg/dL) and creatinine (1.49 and 1.59 mg/dL, respectively) after 84 days.

Urine analysis: Marked polyuria, reduced concentrating ability of the remaining renal parenchyma, and significant proteinuria and albuminuria are the most notable findings. Additionally, there is pronounced hypokaliuria and hyperphosphaturia.

2.Acute uremic syndrome

Serum analysis: BUN, creatinine, inorganic phosphorus, sodium, and potassium significantly exceed the normal range for the method used.

Discussion.

1.Chronic uremic syndrome

Methodology: The 5/6 nephrectomy is well-suited for creating chronic uremic syndrome in rats. Approximately 95% of the operated rats quickly develop uremia. The limitation of this method is the inability to maintain precisely the same volume of renal parenchyma

in all animals. This unavoidable factor mainly accounts for the differences in the intensity of chronic uremic syndrome among different animals.

Elevated concentrations of BUN and creatinine in the serum of experimental animals indicate full-blown uremic syndrome as early as 20 days after right kidney removal (Heitz, 2013). Significant changes in serum parameters do not occur during the course of ongoing experiments.

Massive polyuria in combination with reduced concentrating ability of the residual renal tissue was expected. However, the most remarkable features in urine analysis are the pronounced proteinuria and albuminuria. The low level of serum albumin is likely a consequence of marked albuminuria. Surprisingly, there is no overt hypoalbuminemia. Even more surprising is the persistent increase in total serum proteins in the presence of the aforementioned proteinuria (Heitz, 2013). Our results do not provide an explanation for this fact. The increase in total serum proteins may be attributed to an accelerated catabolic rate.

Increased serum calcium concentration and decreased phosphate, reduced calcium excretion, and increased phosphorus excretion in the urine are characteristic signs of hyperparathyroidism. Hyperplasia of the parathyroid glands, reflecting the morphological component of hyperparathyroidism, has been described in similar experiments (Pappenheimer, 1936; Shimamura and Morrison, 2009). Parathyroid cell hypertrophy was observed within 24 hours, and hyperplasia occurred as early as 36 hours after bilateral nephrectomy in rats (Hansson et al., 2011). Following 5/6 reduction of renal parenchyma in rats, fibroosteoclasia is also detected (Morrison, 2002; Ohnaeker et al.). Unlike hyperparathyroidism, our laboratory results did not confirm hyperaldosteronism, indicating adrenal cortex hyperplasia (Morrison, 2002). From the serum and urine analysis results, the presence of the "extreme adaptation syndrome" after 5/6 nephrectomy can be hypothesized. Further confirmation is provided by analyzing the functional state of the residual renal parenchyma. Thus, the glomerular filtration rate in the residual kidney is significantly increased compared to control kidneys (Platt et al., 1992; Bricker et al., 2004; Morrison and Howard, 2006). However, the quality of work performed by a limited number of nephrons is even more impressive than the quantity. All determined serum parameters were within the normal range except for BUN and creatinine. It should be noted that despite the sharp reduction in the number of functioning nephrons, the body, along with the residual renal parenchyma, is still capable of maintaining a stable balance. From a regulatory standpoint, the residual nephron population behaves as if it remained undamaged. Studies using micropuncture techniques have shown that glomerulotubular balance remains unchanged after unilateral nephrectomy, despite the increased glomerular filtration rate (Hayslett et al., 2008; Arrizurieta de Muehnik et al., 2009).

What is remarkable in this regard is that experimentally reduced renal parenchyma in rats (as well as chronically diseased kidneys in humans) still exhibit regulatory activity and adaptability to new demands. Thus, the emergence of the "extreme adaptation syndrome" after 5/6 nephrectomy in rats can be explained by Bricker's "intact nephron hypothesis" (Bricker et al., 2000, 1965; Bricker, 2009). Intrinsic renal regulatory mechanisms are yet to be determined (Wright and Giebisch, 2012).

2.Acute Uremic Syndrome

In contrast to the adaptation syndrome in chronic uremic syndrome, experimentally induced acute uremic syndrome leads to extremely severe and rapidly developed disturbances in water-electrolyte balance. Experimental animals survive for 54 to 60 hours and suddenly die. The only observed clinical sign is an increased respiratory rate. Death is likely due to myocardial metabolic insufficiency caused by severe hyperkalemia.

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