Journal of Advanced Zoology

ISSN: 0253-7214

*Volume **44** Special Issue **-02 Year 2023** Page 954:959*

MORPHOMETRIC INVESTIGATION OF THE SMALL INTESTINAL MUCOSA OF RATS WITH CHRONIC EXPERIMENTAL RENAL INSUFFICIENCY

Sh.A.Abdulkhakimov

Bukhara State Medical Institute Bukhara, Uzbekistan

Khasanova D.A.

Bukhara State Medical Institute Bukhara, Uzbekistan

N.O.Murodullayeva

Bukhara State Medical Institute Bukhara, Uzbekistan

Article History

Received: 29Aug 2023

Revised: 30Sept 2023

Accepted: 08 Oct 2023

Abstract: In this study, we aimed to simulate and analyze two distinct uremic syndromes in rats: a chronic uremic syndrome induced by performing a 5/6 nephrectomy and an acute uremic syndrome induced by bilateral nephrectomy. The chronic uremic syndrome in rats exhibited several notable characteristics upon examination of serum and urine samples. Specifically, there were substantial increases in blood urea nitrogen (BUN) and creatinine levels, indicative of impaired renal function. Furthermore, these rats displayed biochemical markers consistent with secondary hyperparathyroidism.

Interestingly, the animals with chronic uremic syndrome displayed pronounced symptoms such as severe polyuria (excessive urine production), proteinuria (presence of excess protein in the urine), and albuminuria (abnormal levels of albumin in the urine). Counterintuitively, the total serum protein levels were found to be elevated in this group. This constellation of findings suggests that the experimental chronic uremic syndrome can be characterized as a state of "extreme adaptation," impacting not only the regulatory functions of the remaining renal tissue but also influencing the overall systemic physiology of the animals.

In contrast, the acute uremic syndrome induced by bilateral nephrectomy in the rats represented a severe disorder affecting water and electrolyte balance. This condition had dire consequences, leading to the demise of the experimental animals within a relatively short timeframe of 50 to 60 hours.

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

Introduction. Uremic syndrome represents a complex condition that exerts widespread effects on the entire body. Research suggests that a disruption in cellular metabolism is the underlying cause of functional impairments in multiple organs (Bergstrom & Bittar, 2009; Teschan, 2010). Several studies have indicated that oxidative phosphorylation within mitochondria may bear the brunt of this impact (Morgan et al., 2003; Lennar, 2008; Heitz et al., 2011). Nevertheless, the

precise pathogenesis of this disorder remains incompletely elucidated. Several pathogenetic mechanisms have been postulated, including:

1. An imbalance in electrolytes, with particular emphasis on calcium and magnesium ions (Kimich & Lassmussen, 2009).
2. Disturbances in the acid-base balance leading to acidosis (Lellman, 2012).
3. The formation of "uremic toxins" (as reviewed by Teschan, 2010; Dobbelstein, 2011).

Renal insufficiency can be induced experimentally in rats through methods such as a substantial reduction in the total number of functioning nephrons, bilateral nephrectomy, or a 5/6 reduction in renal parenchyma. These approaches are grounded in two critical considerations:

1. The extent of loss of functional renal parenchyma is the pivotal determinant of renal dysfunction, irrespective of the underlying disease (Bricker et al., 2015).
2. In rats aged beyond 2 months, there is no further increase in the nephron count (Bonvale et al., 2002).

The experiments conducted herein aimed to assemble a comprehensive set of functional parameters that can effectively characterize experimental uremic syndrome in rats. These parameters will subsequently be correlated with the outcomes of both stereological and biochemical analyses of liver tissue. Additionally, the data will be examined alongside information regarding calcium and strontium metabolism in rats afflicted with uremic syndrome, as presented in the work of Remagen et al.

In essence, the primary objective of these experiments was to gather a wealth of information pertaining to the functional parameters that define uremic syndrome in rats. These insights will then be juxtaposed against the results derived from the stereological and biochemical assessments of liver tissue, as well as data concerning calcium and strontium metabolism in rats affected by this syndrome, as documented in the research conducted by Remagen and colleagues.

Materials and methods. 1. For the induction of chronic uremic syndrome, we utilized a modified technique, as outlined in the works of Chanutin and Ferris (1982), Platt et al. (1952), and Morrison (2006). Specifically, in 44 male Wistar inbred rats (weighing between 175-190 grams, sourced from Hoffman-La Roche, Ltd, Basel), we performed a subcutaneous implantation of the left kidney into the dorsal region, utilizing a lateral incision while the animals were under ether anesthesia. To achieve a reduction in the parenchymal volume to one-third of its original size, we resected both poles of the kidney. Subsequently, after a recovery period of 8-10 days, we proceeded to remove the entire right renal organ. As a point of comparison, in a control group consisting of 21 animals (weighing between 175-195 grams), we performed unilateral nephrectomy.

2. Acute uremic syndrome was induced by conducting bilateral complete nephrectomy in 8 male Wistar rats (with body weights ranging from 190-210 grams). To provide a baseline for comparison, we included six rats that underwent sham surgery (with body weights in the same range) and three untreated rats (weighing between 200-210 grams) as controls. All animals were housed in plastic cages, with two rats per cage. They were supplied with standard laboratory feed (Altromin-t~) and had unrestricted access to drinking water provided via plastic bottles.

3. In the serum analysis, we conducted the following measurements on 12 animals subjected to 5/6 nephrectomy, 21 animals in the control group, as well as on all experimental animals with bilateral nephrectomy and the corresponding control group: blood urea nitrogen (BUN), creatinine, phosphates, chlorides, albumin, total protein, alanine aminotransferase (SGPT), and alkaline phosphatase, utilizing an autoanalyzer. Additionally, we determined the levels of sodium, potassium, and total calcium through flame photometry using an Eppendorf photometer. Blood

samples were collected from the retroocular plexus at specific time points, including the 20th, 40th, and 84th day after the removal of the right kidney, as well as 48 hours post-bilateral nephrectomy.

4. In the urine analysis phase, we gathered urine samples from three rats that had undergone 5/6 nephrectomy and three control animals over a three-day period (ranging from the 72nd to the 74th day following the removal of the right kidney). This analysis encompassed the measurement of 24-hour urine volume, osmolality, and pH, in addition to the previously mentioned serum determinations. To conclude the experiment, the animals were humanely euthanized through decapitation on the 85th day after the removal of the right kidney and 48 hours post-bilateral nephrectomy.

Results.

1. Chronic Uremic Syndrome

Serum Analysis: The presence of chronic uremic syndrome was convincingly confirmed through serum analysis, with a persistent and notable elevation observed in both blood urea nitrogen (BUN) and creatinine levels, surpassing the normal range for the applied measurement methods. Notably, statistically significant disparities were identified between the experimental group and the control group concerning calcium, potassium, and chloride levels. Total protein levels were elevated, whereas inorganic phosphorus and albumin levels exhibited reductions. Furthermore, when comparing values obtained on the 20th and 40th days following the removal of the right kidney, there was a modest increase in BUN levels (20 days: 77.6 mg/dL, 40 days: 83.2 mg/dL) and creatinine levels (1.49 mg/dL and 1.59 mg/dL, respectively) after an 84-day period.

Urine Analysis: The most prominent findings in urine analysis were marked polyuria, indicative of excessive urine production, a diminished concentrating capacity of the remaining renal parenchyma, and significant occurrences of proteinuria and albuminuria. Additionally, there was a notable presence of hypokaliuria (reduced potassium excretion) and hyperphosphaturia (increased phosphorus excretion).

2. Acute Uremic Syndrome

Serum Analysis: In cases of acute uremic syndrome, serum analysis revealed significant deviations from normal ranges for the method employed. BUN, creatinine, inorganic phosphorus, sodium, and potassium levels exhibited substantial increases, signifying a clear departure from physiological norms.

Discussion.

1. Chronic uremic syndrome

Methodology: The 5/6 nephrectomy procedure has proven to be a highly effective method for inducing chronic uremic syndrome in rats, with approximately 95% of the operated rats rapidly developing uremia. However, one limitation of this technique is the inability to precisely maintain an identical volume of renal parenchyma in all animals. This unavoidable variability is a significant contributing factor to the differing degrees of chronic uremic syndrome intensity observed among different animals.

Elevated concentrations of blood urea nitrogen (BUN) and creatinine in the serum of experimental animals serve as early indicators of full-blown uremic syndrome, detectable as soon as 20 days following the removal of the right kidney (Heitz, 2013). Importantly, significant changes in serum parameters do not manifest over the course of ongoing experiments.

The anticipated outcomes of massive polyuria, coupled with reduced concentrating ability of the remaining renal tissue, were observed. However, the most remarkable findings in urine analysis

were the pronounced occurrences of proteinuria and albuminuria. Surprisingly, despite marked albuminuria, there is no evident hypoalbuminemia. Even more surprising is the persistent increase in total serum proteins in the presence of the aforementioned proteinuria (Heitz, 2013). The precise explanation for this phenomenon remains elusive, but it may be attributed to an accelerated catabolic rate.

Notably, increased serum calcium levels and decreased phosphate levels, along with reduced calcium excretion and increased phosphorus excretion in the urine, are indicative signs of hyperparathyroidism. Similar experiments have reported the presence of hyperplasia in the parathyroid glands, which reflects the morphological component of hyperparathyroidism (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). In addition to hyperparathyroidism, experiments involving 5/6 reduction of renal parenchyma in rats have detected fibroosteoclasia (Morrison, 2002; Ohnaeker et al.). However, unlike hyperparathyroidism, laboratory results did not confirm hyperaldosteronism, indicating adrenal cortex hyperplasia (Morrison, 2002).

From the results of serum and urine analysis, the presence of an "extreme adaptation syndrome" following 5/6 nephrectomy can be postulated. Further confirmation is provided by assessing the functional state of the residual renal parenchyma. Remarkably, the glomerular filtration rate in the remaining kidney is significantly increased compared to control kidneys (Platt et al., 1992; Bricker et al., 2004; Morrison and Howard, 2006). However, what is even more impressive than the quantity of functioning nephrons is the quality of their performance. All determined serum parameters, except for BUN and creatinine, remained within the normal range. It is worth noting that despite the drastic reduction in the number of functioning nephrons, the body, in conjunction with the residual renal parenchyma, retains the capacity to maintain a stable balance. From a regulatory standpoint, the remaining nephron population behaves as if it had not suffered any damage. Studies employing micropuncture techniques have demonstrated that glomerulotubular balance remains unaltered after unilateral nephrectomy, despite the increased glomerular filtration rate (Hayslett et al., 2008; Arrizurieta de Muehnik et al., 2009).

What is particularly noteworthy in this context 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. This phenomenon can be explained by Bricker's "intact nephron hypothesis" (Bricker et al., 2000, 1965; Bricker, 2009), although the precise mechanisms of intrinsic renal regulatory processes are yet to be fully elucidated (Wright and Giebisch, 2012).

2.Acute Uremic Syndrome

In stark contrast to the adaptation syndrome observed in chronic uremic syndrome, the experimentally induced acute uremic syndrome presents with exceptionally severe and rapidly progressing disruptions in water-electrolyte balance. In this scenario, experimental animals exhibit a remarkably short survival period, typically ranging from 54 to 60 hours, after which they experience sudden and unexpected demise. Notably, the sole clinical indicator of this impending catastrophe is an elevated respiratory rate.

The primary cause of death in this context is believed to be myocardial metabolic insufficiency, which is precipitated by severe hyperkalemia, a condition characterized by excessive levels of potassium in the bloodstream.

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