A SURGICAL RAT MODEL FOR CHRONIC RENAL FAILURE

by

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Approved by:

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To

My husband, Yaw-Boadu, for his love, patience, and encouragement.

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INTRODUCTION

In recent years, the life of the uremic patient has been lengthened with advent of long-term, intermittent dialysis and transplantation. Between 10,000 to 18,000 patients are undergoing dialysis, with male patients outnumbering women patients by approximately two to one (1). However, patients with chronic renal failure experience a host of problems related to intermediary metabolism whose solution requires attention.

Growth retardation in children, weight loss, and other evidence of wasting are commonly observed in patients with chronic renal failure (2-4). In the presence of significant renal disease, the ability to sustain a viable pregnancy decreases, and pregnancy in patients with pre-existing parenchymal disease may further compromise renal function (5). Chronic renal failure is commonly associated with glucose intolerance characterized by insulin resistance in adult onset diabetes mellitus (6). Metabolic acidosis, intestinal malabsorption of amino acids and minerals, alterations in vitamin D_3 metabolism as well as increased circulating levels of parathyroid hormone, uremic toxins, and abnormal behavioral changes attend chronic renal failure (6-12).

Anemia remains a persistent problem in patients with renal insufficiency and can contribute to the mortality of patients on maintenance hemodialysis (13). Fatigue, exortional dyspnea, palpitations, headache, irritability and paresthesias as well as myocardial hypoxia are all potential consequences of anemia. Transfusions are known to depress erythropoiesis and continue to carry the risk of transmitting hepatitis, producing iron overload and precipitating heart failure (14).

Clinical evidence suggest that cardiovascular disease is the leading cause of deaths in patients receiving long-term dialysis (1,15-16). It is responsible for 8 to 26 percent of deaths in renal transplant recipients (1, 16-18), being second only to infection as a cause of death (19). The cardiovascular disease seen in these patients is related to risk factors such as hypertension, hyperuricemia, fluid overload, anemia, glucose intolerance, lipid and mineral abnormalities associated with uremia (20-22). Elevated triglycerides frequently accompany chronic hemodialysis, and may be a factor in the development of premature arteriosclerosis (23-25).

Several animal models have been used to investigate chronic uremia (26-28). The animals are usually made uremic by exposure to chemicals (29-30). Although these models resemble kidney failure in humans to some extent, the chemicals may affect other organs such as the liver. The effect of chemicals on the liver may mask the metabolic changes associated with kidney failure. There are several immunological models of kidney failure (31-33); however, nephrotic syndrome commonly occurs in these models and may alter nutrition and amino acid and protein metabolism.

Nephrectomy of one kidney and sub-total surgical ablation or infarction of the other kidney causes tubular necrosis. Irradiation or electrocauterization of the remaining renal tissue prevents the parenchyma from hypertrophying and therefore, maintains the necrosis (34-36). Since this diminution of the kidney by surgery affords the possibilities of quantification, it is the technique most often chosen.

This study was undertaken to develop an experimental rat model in this laboratory for chronic renal failure. Development of a successful model would enable studies to be carried out regarding nutritional factors affecting lipid, protein and carbohydrate metabolism in chronic renal failure.

REVIEW OF LITERATURE

Surgical Approach

Uremia, a general term applied to the syndrome arising from the failing function of the kidney, refers to the "retention of urea and other urinary constituents in the blood". Therefore, blood urea nitrogen (BUN), urea or serum creatinine determinations provide an index of the degree of uremia. Since the early experimental work on partial nephrectomy by Tuffier (37) in 1889, a multitude of detailed studies have been conducted on renal function in various animal models. The degree of uremia produced in such kidney insufficient animals is listed in Table 1. Table 2 contains the pooled control values of these indexes. Following is a review of methods used to induce experimental renal failure.

Models with portions of the renal mass ligated. Accumulated reports show that uremia is accompanied by abnormal cardiac function and histology both in humans and animals (15-16). This finding intrigued Williams and Luft (24) into investigating whether the alterations are secondary to the associated abnormalities such as hypertension and atherosclerosis. They studied the effects of surgically induced uremia in the rat on myocardial lipid and carnitine esters. Uremia was produced in 225-250 g rats by five-sixths nephrectomy via a two-stage procedure. Matress sutures were placed through the upper and lower poles of the left kidney, and the poles were excised, which left only a hilar remnant. Five to seven days later, the right kidney was excised. In this model, serum creatinine (1.3 ± 0.3 vs 3.9 ± 1.2 mg/dl) and serum urea nitrogen (19 ± 5 vs 147 ± 57 mg/dl) concentrations were increased threefold

TABLE 1

Surgical models for reduced renal function.

INVESTIGATOR	Animal Species	Surgical Approach	Degree of Nephrectomy	INDEXES OF RENAL FUNCTION						
				Creatine Clearance	Urea Clearance	Glomerular Filteration Rate	BUN	Serum Urea	Serum Creatinine	Uric Acid
				ml/mn	ml/mn	ml/mn	mg/dl	mg/dl	mg/dl	mg/dl
Nitzan et al (5)	Rats	Ablation	5/6	—	—	—	44.3±2.1	—	1.27±0.03	—
Espinel (7)	"	"	75%	—	—	0.23±0.1	—	—	—	—
Wilson & Honrath (9)	"	"	5/6	—	—	—	36±2.0	—	—	—
Schiffl & Binswanger (10)	"	"	"	—	—	—	—	107±14.0	—	—
William & Luft (24)	"	Ligation	"	—	—	—	147±57	—	3.9±1.2	—
Hartenbower & Coburn (27)	Chicks	"	"	—	—	—	—	—	—	11.9±1.11
Ormrod & Miller (28)	Rats	Ablation	Severe	—	—	—	—	200	2.8	—
	"	"	Moderate	—	—	—	—	100	1.9	—
	"	"	Mild	—	—	—	—	40	0.8	—
	"	"	'Sham'	—	—	—	—	18	0.5	—
Silva & Albuquerque (29)	"	"	5/6	—	—	—	—	124±27.7	1.30±0.24	—
Sterner et al (35)	"	Ablation/or Irradiation	"	0.63±0.26	—	—	—	—	3.68±0.8	—
Boudet et al (36)	"	Ablation/or Electrocoagulation	"	1.14±0.4	—	—	—	—	5.9±0.79	—
Baati et al (38)	"	Ligation	"	—	—	1.4±0.75	56±4.0	—	—	—
Bank & Aynedjian (39)	Rats	Ligation	5/6	—	—	—	77.6±7.4	—	—	—
Siassi et al (40)	"	"	"	0.5±0.4	0.1±0.01	—	55.1±20.1	—	1.6±0.7	—
Russell & Avioli (41)	"	"	"	—	—	—	56.0±2.6	—	1.24±0.05	—
Bagdade et al (43)	"	Ablation	"	—	—	—	26±3.0	—	—	—
Soman & Pelig (44)	Group I Rats	"	"	—	—	—	30±2.0	—	2.20±0.22	—
	Group II Rats	"	"	—	—	—	46±1.0	—	4.21±0.73	—
Salusky et al (46)	Rats	"	"	—	—	—	84±8.9	—	4.41±0.17	—
Saeed et al (47)	"	"	"	—	—	—	—	99.6±26.0	—	—
Ellis et al (48)	"	"	"	—	—	—	89±9.4	—	1.16±0.11	—
Latteri et al (49)	"	"	"	—	—	—	—	192±42.0	1.03±0.20	—
Schulz et al (50)	Rabbits	"	"	—	—	—	79.0±1.7	—	—	—
Anagnostou et al (51)	Rats	"	"	—	—	—	120±33.0	—	—	—
Weisbrode & Capen (52)	"	"	"	—	—	—	65.0±5.0	—	—	—
Hauck et al (60)	"	Ablation/or Irradiation	8/10	0.92±0.32	—	—	—	—	—	—
Lin et al (62)	"	Ablation/or Electrocoagulation	5/6	—	—	—	112±20.0	—	—	—
Kamstrup et al (63)	Rabbits	"	"	—	—	—	—	168	5.5	—
Grimmel et al (67)	Rats	"	"	—	—	—	69.0±12.6	—	—	—

TABLE 2

Pooled control values of uremic indexes

Investigator (Reference Number)	Creatinine Clearance	Urea Clearance	Glomerular Filteration Rate	BUN	Serum Urea	Serum Creatinine	Uric Acid
	ml/min	ml/min	ml/min	mg/dl	mg/dl	mg/dl	mg/dl
(9,36,60)	0.69±0.08						
(8,9)		0.7±0.13					
(7)			0.2±0.02				
(5,7-10,24 29,38-40,43 44,46-52,62 64)				18.5±0.9			
(10,29,35, 46,49)					39.9±3.1		
(5,9,21,29, 40,44,48,49)						0.73±0.09	
(41)							10.8±0.05

and sevenfold, respectively over control animals. The investigators used this uremic rat model to demonstrate that chronic uremia does not result in impairment of the enzymes of intermediary metabolism in the heart.

Bastl et al. (38) succeeded in producing a noticeable renal insufficiency in Sprague-Dawley rats, weighing 250-400 g, by suturing the upper and lower poles of the kidney mass and excising the ischemic portions made by the tightened ligature. The procedure resulted in the removal of 80% of the nephron population and a significant reduction in glomerular filteration rate (0.291 ml/min/100 g body wt.), approximately 30-40% of control value (1.076 ml/min/100 g body wt.) and a reduction in renal plasma flow (0.4244 ± 0.0588 vs 1.375 ± 0.075 ml/min/100 g body wt.), and significantly increased BUN (56 ± 4 mg/dl in experimental) as compared to control animals 27 ± 1 mg/dl, ($P < 0.001$).

Bank and Aynedjian (39) removed the right kidney and ablated the cephalad half of the left kidney of male Sprague-Dawley rats, weighing 225-350 g, by silk ligature. This left much of the ventral surface of the kidney undisturbed to permit micropuncture collections in order to determine the site along the nephron responsible for increased potassium excretion. The blood urea nitrogen increased from a control level of 17.8 to 77.6 mg/dl with a large increase in tubular fluid potassium content between the end of the distal tubule and in the final urine in the nephrectomized rats. Their findings suggested that the collecting duct is the major site of the regulation of urinary potassium excretion in normal rats and is responsible for the adaptation to nephron loss by the remnant kidney.

Wilson and Honrath (9) investigated the mechanisms involved in the capacity of the diseased kidney to retain sodium under circumstances of gradual reduction of sodium intake. They performed a three-quarter nephrectomy in male Sprague-Dawley rats (weighing 250-375 g) by totally removing the right kidney and excising the ligated upper and lower poles of the left kidney. The nephrectomized rats showed an increase in BUN level of 38 $\pm$ 4 mg/dl as compared to the normal control value of 15 $\pm$ 2 mg/dl. The glomerular filteration rate was 32% of sham-operated normal rats. Urine flow and fractional excretion of water, sodium, and potassium were all significantly higher in the three-quarter nephrectomized group than in the non-operated rats. Data obtained supported the concept that the circulating factors in renal insufficiency have direct inhibitory effect on tubular reabsorption of sodium and potassium. In general, the surgical approach of ligating portions of the renal mass before incision is accompanied by little hemorrhage. which eliminates a problem which can aggravate the anemia of renal failure (13).

Models with ligated renal arteries or ureter. In a study dealing with chronic renal insufficiency in rats, Wang and co-workers (8) reported the results obtained after feeding different levels of proteins to rats (180-200 g) made uremic by ligation of two-thirds to three-forths of the primary and secondary divisions of the left renal artery, with contralateral nephrectomy. The surgery resulted in a significant reduction in urea and creatinine clearances. In the experimental group urea clearance was 0.3 $\pm$ 0.1 ml/min vs 0.8 $\pm$ 0.3 ml/min for controls ($P < 0.001$). Creatinine clearance was 0.3 $\pm$ 0.4 ml/min in the experimental group compared with 0.7 $\pm$ 0.3 ml/min for controls ($P < 0.05$).

Likewise, plasma urea nitrogen and creatinine concentrations were significantly ($P < 0.001$) elevated above pair-fed controls. BUN in the experimental group was 55.1 ± 20.1 vs 18.9 ± 3.1 mg/dl in controls. Serum creatinine concentration was 1.6 ± 0.7 mg/dl in the uremic rats compared with 0.9 ± 0.3 mg/dl in the controls. Uremic rats gained less weight ($P < 0.02$) and had a lower protein efficiency ratio. The observations of this study indicated that both uremia and reduced protein intake may affect growth and amino acid metabolism. Uremic rats utilized protein less efficiently, which indicates that marked reductions in protein intake may be particularly harzadous to the nutritional status of the uremic patient.

In an attempt to study the pathological state that modifies the levels of plasma tryptophan and alters brain serotonin metabolism, Siassi and colleagues (40) induced chronic renal failure in rats (160-180 g) by partial ligation of the left renal artery followed by contralateral nephrectomy, and observed a significant rise in plasma urea nitrogen (58.6 ± 16.5 vs 17.9 ± 3.0 mg/dl) with a significantly decreased urea clearance (0.16 ± 0.06 ml/min, $P < 0.001$). This study demonstrated that uremic stress, in addition to altering plasma tryptophan levels, also affected brain serotonin turnover; a condition which has been implicated in neurological symptoms often associated with the uremic state.

Russell and Avioli (41) evaluated the effect of chronic renal disease on bone matrix and mineral metabolism in six-week old Holtzman rats made uremic by ligation of two of the left renal arteries, followed by unilateral nephrectomy of the right kidney. BUN was 56.0 mg/dl in the experimental group and 24.4 mg/dl in the pair-fed controls ($P < 0.001$).

Serum creatinine was 1.24 mg/dl in the uremic group compared to 0.56 mg/dl in controls. These uremic rat models allowed demonstration of intestinal malabsorption of calcium in uremia. Since patients with chronic renal failure frequently exhibit maturation defects in both collagen and mineral metabolism, the investigators believed that this uremic rat model could be particularly important in studying the relationship of these findings to 1,25-di-OH-cholecalciferol metabolism.

Day old chicks were utilized by Hartenbower and Coburn (27) in a study of chronically reduced renal function. By ligation of the right ureter just proximal to its junction with the cloaca (figure 1), the function of one kidney was completely eliminated and the functional mass of the other was reduced by two-thirds when the left ureter and renal vein were ligated with a single suture near the middle of the left kidney. The method resulted in elevation of plasma concentrations of uric acid (11.9 $\pm$ 1.11 mg/dl, experimental vs 6.2 $\pm$ 0.53 mg/dl, control), the major end product of protein metabolism in avian plasma. They claimed that the procedure is not only inexpensive, quick and simple, but that the impairment of the renal function is reproducible with a high survival rate.

Criticisms could be raised against the techniques for the study of renal failure induced by a combination of ligation of most of the renal mass or ligation of the renal arteries. This approach may introduce reactions such as reabsorption of the necrotic tissue and uncompensated metabolic acidosis which could mask the true effects of chronic kidney failure. The ligation, or constriction of the renal mass in particular, may introduce undesirable factors such as secondary systemic hypertension due to ischemia of the kidney tissue (42).

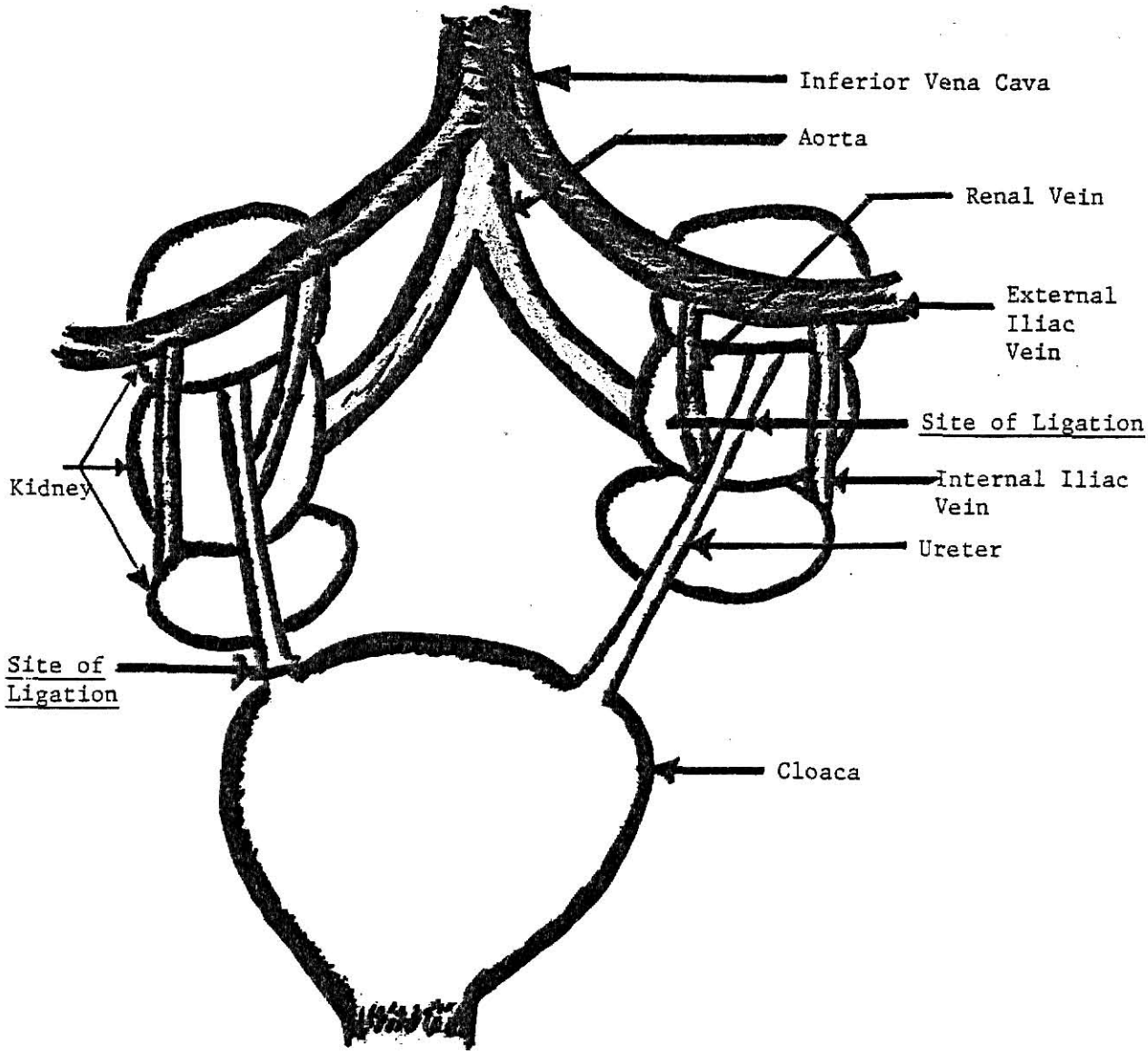

Fig 1. Schematic drawing of the urinary system of the chick, with site of surgical ligation indicated (27).

Models with partially ablated renal parenchyma. In an effort to avoid the undesirable factors associated with combining ligation and ablation to induce renal failure, many investigators turned to a simple resection of portions of the kidney mass. In such an effort, Bagdade et al. (43) induced chronic uremia in Sprague-Dawley rats (100-150 g) by surgically removing the upper and lower poles of the left kidney, followed by a right nephrectomy a week later. All controls were sham operated. Four-weeks following partial nephrectomy, significantly higher serum triglycerides (141 $\pm$ 52 mg/dl in experimental rats compared to 83 $\pm$ 5 mg/dl in the controls, $P < 0.01$) and BUN (26 $\pm$ 5 mg/dl in experimental as compared to 11 $\pm$ 2 mg/dl in the controls, $P < 0.01$) were observed in the experimental animals. Persistent abnormalities in triglyceride transport were observed which were similar to those previously described in patients with a more profound degree of azotemia. This uremic animal model may provide useful insights applicable to the study of plasma lipid transport in renal failure in man.

After the removal of 70% (group 1) and 90% (group 2) of the renal parenchyma in adult Sprague-Dawley rats weighing 200-300 g, Soman and Felig (44) noted a moderate elevation of BUN (30 $\pm$ 2 mg/dl in group 1, and 46 $\pm$ 3 mg/dl in group 2) and high serum creatinine level (2.20 $\pm$0.22 mg/dl in group 1 and 4.21 $\pm$ 0.73 mg/dl in group 2); hyperglycemia; hyperinsulinemia and hyperglucagonemia in these uremic animal models. All these abnormalities compare well with the major characteristics of carbohydrate intolerance in uremic patients (45).

In testing the effect of various protein diets with regard to growth and survival, Salusky et al. (46) rendered male Wistar weanling rats

uremic by a two-stage surgical procedure: Four-fifths of the left kidney was resected followed by a total right nephrectomy four days later. After one week recuperation period, the nephrectomized rats were matched and distributed into three groups according to their serum creatinine. Rats were fed three different diets: diet I containing 7.5% protein, diet II, 7.5% protein plus 1% EAA (essential amino acids) and diet III containing 14% protein. The use of protein deficient diets complemented with EAA, promoted satisfactory growth and reduction in serum urea nitrogen from 84 $\pm$ 8.9 to 40.8 $\pm$ 2 mg/dl. The data suggested that the diet used and particularly its protein content are critical for the deterioration of renal function of rats with chronic renal failure.

Saeed and colleagues (47) studied the effects of five-sixths nephrectomy on the concentration of insulin and glucose in the blood. Adult albino rats of the Wistar strain were made renal insufficient by removing the whole right kidney and the upper and lower thirds of the left kidney using wedge-shaped incisions; sham operation consisted of mobilization of the kidneys from the surrounding fascia. The five-sixths nephrectomy resulted in a marked increase in the level of urea in the plasma (99.6 $\pm$ 26.0 mg/dl vs 36.0 $\pm$ 2.4 mg/dl), without any significant changes in the concentrations of insulin or glucose in the circulation. They suggested that about one-sixth of the renal mass is sufficient to deal with the degradation and excretion of insulin.

Nitzan et al. (5) concluded from their studies of pregnant Sprague-Dawley rats that the bipolar removal of three-forths of the kidney substance causes a rise in BUN from 17.6 mg/dl to 44.3 mg/dl, loss of appetite, intrauterine growth retardation and mild alkalosis. Ormrod and Miller (28) also concluded from their studies on dark agouti rats that

the ablation of 76-80% of the kidney substance, causes loss of appetite and wasting, with a survival rate of 75-80%.

Increasing reports of progressive abnormalities of bone and calcium in patients with chronic renal failure, led Ellis et al. (48) to explore these derangements in an animal model. Sprague-Dawley rats 200-250 g received a unilateral partial bipolar nephrectomy, followed by contralateral total nephrectomy a week later. This resulted in a moderate uremia (BUN of 53-89 mg/dl) and serum creatinine level of 0.7-1.2 mg/dl, with impaired growth in the uremic rats.

Schiffl and Binswanger (10) subjected male albino rats (obtained as 70 g weanlings) to either a five-sixths nephrectomy in a two-stage procedure or sham operation. The partial nephrectomy involved the removal of two-thirds of the left kidney, followed by a total right nephrectomy seven-days later. Plasma urea was elevated (107 $\pm$ 14 mg/dl) in all subtotally nephrectomized animals. Their data suggested that chronic uremia can decrease calcium ATPase activity and impair the rise of the enzyme after calcium deprivation. Letteri and co-workers (49) also demonstrated less bone growth in sub-totally nephrectomized rats. Young Sprague-Dawley rats approximately ten-weeks old, weighing between 200 and 250 g, underwent a unilateral partial bipolar nephrectomy, followed one-week later by a contralateral nephrectomy. They explained that though the nephrectomized rats provide a convenient model for studying disordered mineral metabolism in renal failure, nonetheless, this model differs in many respects from the insidius development of renal insufficiency most commonly encountered in man.

Espinel (7) demonstrated in uremic rat models that as renal failure progresses, dietary salt can determine the level at which plasma bicarbonate

is maintained. He produced chronic renal failure in adult female Sprague-Dawley rats by sequential partial nephrectomies in three stages separated by an interval of at least 2 weeks. Stage I consisted of the removal of approximately 75% of the left kidney; stage II, 75% of the right kidney, and stage III, removal of the remnant right kidney. These rats were fed diets containing different levels of salt. He claimed that the operative removal of 5/6 of the renal mass in three-stages resembles the natural pathogenesis of renal failure, the onset of which is gradual in humans.

Using this rat model, he was able to demonstrate that a gradual reduction of dietary salt in direct proportion to the fall in glomerular filteration rate, may lead to a partial correction of bicarbonate deficit, which may have a therapeutic application in the management of metabolic acidosis of chronic renal failure.

Schultz et al. (50) made rabbits chronically uremic by removing five-sixths of the kidney mass. In the first operation, two-thirds of one kidney was ablated followed by a total nephrectomy of the contralateral kidney 14 days later. The second operation was followed by a sharp increase of BUN (79 $\pm$17 mg/dl). The azotemic state led to a significant rise in the number of CFU-E (colony forming unit-erythroid, a cell which responds to serum erythropoietic stimulating factor (ESF) in the bone marrow). This led to the conclusion that ESF deficiency may not be a major contributing factor in the early stages of anemia of renal insufficiency, but rather a decreased response of the bone marrow to the ESF. There may be a blockade of further differentiation of the erythroid precursors, a condition of particular importance in most patients.

In a study of chronic renal failure in rats, Anagnostou et al. (51) found that the operative removal of five-sixths of the kidney mass, causes a rise in BUN level (120 ± 33 mg/dl) with a fall in hematocrit (30%), and a fall in erythropoietin production to a barely detectable level in the uremic rats. Rats were made uremic following the two-stage procedure. The upper and lower poles of the left kidney were removed through a midline abdominal incision, leaving the pelvis and the hilium intact. In this manner, two-thirds of the left kidney was excised during the first stage. One week later, the right kidney was removed, leaving the animal with only one-sixth of the original renal mass. They claimed that the rats operated on in this way, remained uremic for several months, and provided a potentially useful mode for studying the causes of the anemia of chronically uremic animals.

Silva and Albuquerque (29) utilized uremic rat models to study renal insufficiency. Partial nephrectomy was performed by surgical excision of the upper and lower poles, as well as, a great part of the remaining parenchyma. Renal tissue left was mainly medulla and its corresponding anterior and posterior layers of cortical tissue (figure 2). The premise was that the surgical ablation of 70-80% of the kidney tissue in rats does not produce anemia, hypertension and uncompensated metabolic acidosis even when prolonged renal insufficiency results. They concluded that this experimental rat model will be extremely useful for the studies of chronic effects of uremic chemical toxemia on organs and systems.

Weisbrode and Capen (52) rendered male Sprague-Dawley rats (350 g) uremic by one stage 5/6 nephrectomy. Using a paracostal approach, the anterior and posterior thirds of the left kidney and entire right kidney were removed. Half of the uremic rats were given vitamin D and the other

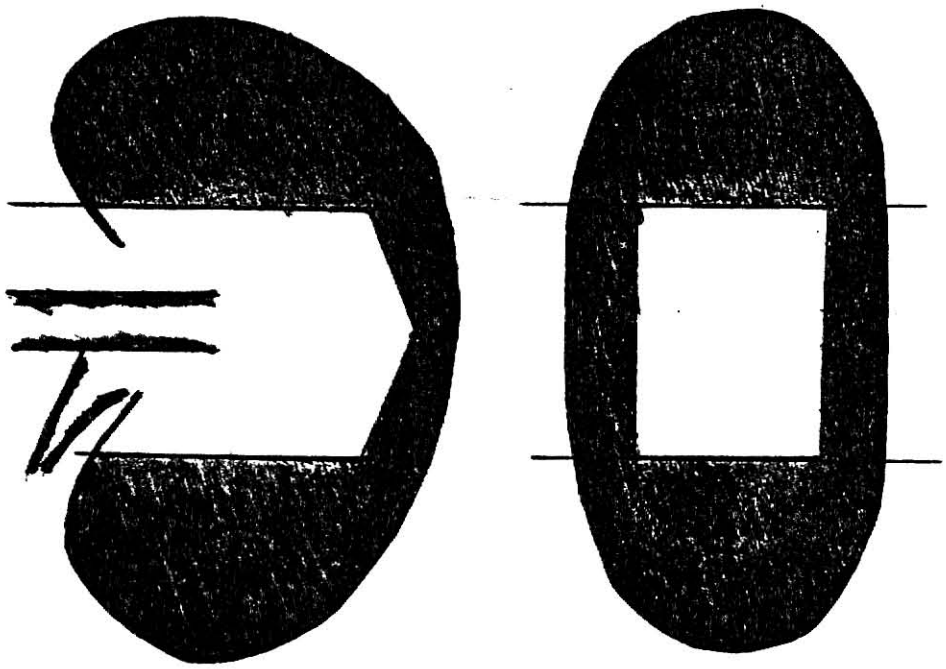

Fig 2. Induction of moderate uremia. The dark areas represent resected tissue. The production of 'moderate' uremia is carried out by surgical excision of the upper and lower poles, as well as, a great part of the remaining parenchyma (29).

half received a placebo. The experimental rats given vitamin D demonstrated a BUN concentration of 65 $\pm$ 5.0 mg/dl, as compared to uremic rats given a placebo (28.0 $\pm$ 2.0 mg/dl, $P < 0.001$). The data inferred that hypercalcemia that develops in renal failure, may be ascribed to selective resorption of cortical bone and impairment of renal calcium excretion. This finding is of particular interest since hypercalcemia may be a clinical problem in uremic patients treated with vitamin D metabolites.

Ormrod and Miller (28) described a method of producing varying degree of stable uremia (figure 3). Three-levels of uremia were attained using a controlled resection of renal tissue. A 'severe' state was produced by resecting both the anterior and posterior poles and then removing one third of the remaining cortical tissue from the exterior lateral aspect of the kidney; followed by removal of 2 mm slices from the dorsal and ventral surfaces of the remnant kidney, leaving only 24% of the intact kidney. Bleeding was controlled by the application of a solution of thrombin to the cut surfaces. Seven days later the right kidney was removed after ligation of the blood vessels and ureter. 'Moderate' uremia was produced by removing the poles and top of the kidney, leaving the dorsal and ventral surfaces intact. The tissue remaining represented forty percent of the intact kidney. The right kidney was resected seven days later. 'Mild' uremia was produced by removing only the right kidney. Sham operations were carried out by removing thin slices of cortical tissue from the surface of the kidney after decapsulation. Seven days later the right kidney was surgically exposed and the capsule, fat and adrenal dissected free and replaced into the abdominal cavity. Rats with 'mild' 'moderate', and 'severe' uremia had serum urea of 40-80 mg/dl, 100-200 mg/dl and over 200 mg/dl, respectively. Likewise, levels of serum creatinine in

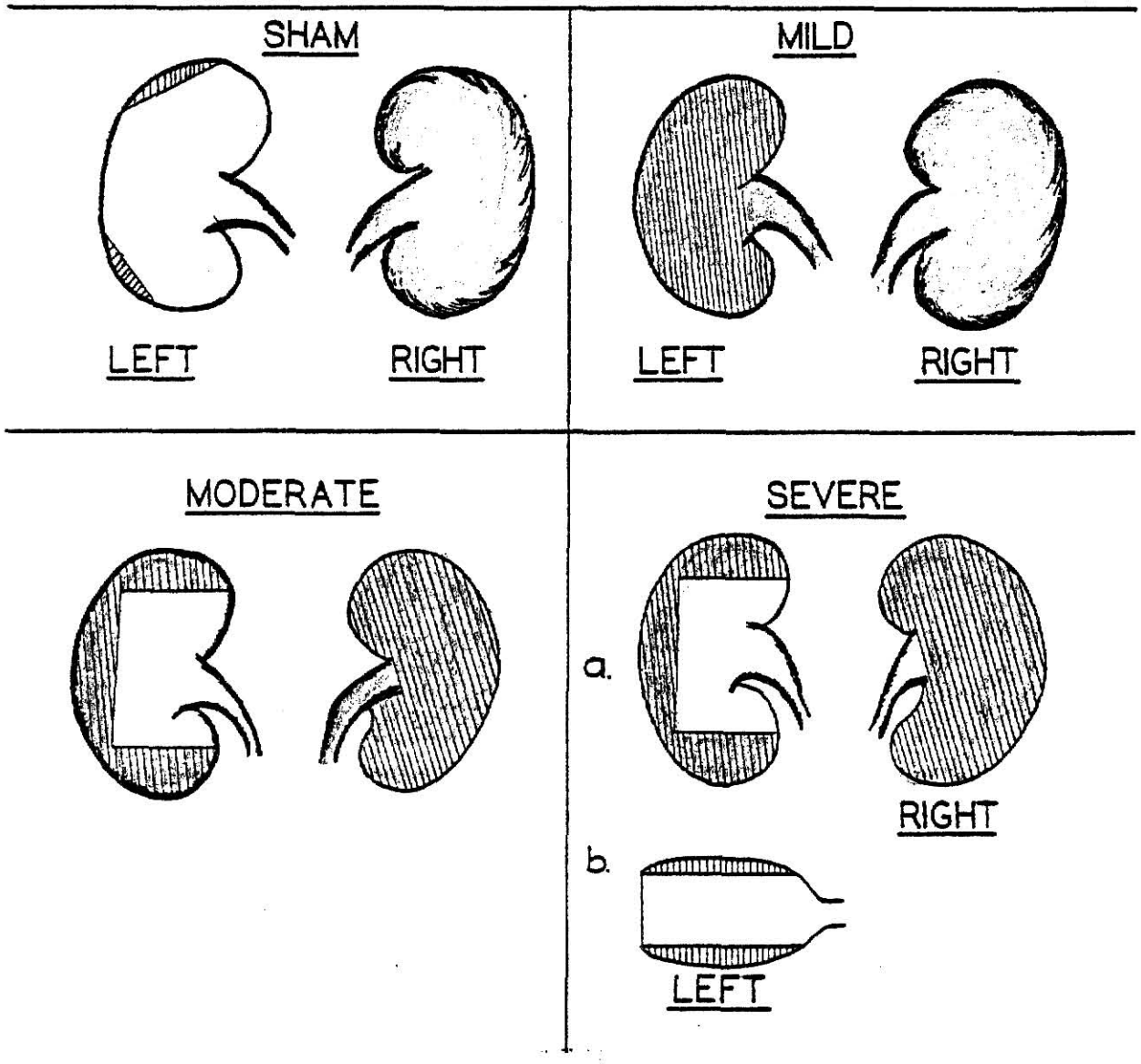

Fig 3. Induction of uremia. The hatched areas represent resected tissue. The production of 'severe' uremia is carried out as a two-step procedure. Tissue is first removed from the poles and lateral surface (a) followed by removal of dorsal and ventral tissue (b) (28).

these different states of uremia were increased from 0.8 - 1.1 mg/dl, 0.8 -2.5 mg/dl to over 3 mg/dl. The investigators inferred that this procedure provides a means for the standardization of the methods of induction of uremia by partial ablation of the renal parenchyma.

Hypertrophy of the remnant kidney tissue. The induction of renal insufficiency by the surgical approach has been characterized by hypertrophy of the remaining renal tissue with an increased mean nephron blood flow and a change in blood flow distribution. For this reason the experimental animals become only slightly or moderately uremic (27,37,49). Hartenbower and Coburn (27) reported hypertrophy of the functional portion of the remnant kidney function in chicks. Ellis et al. (48) and other investigators (49,51) have demomstrated a fall in blood urea nitrogen (BUN), serum creatinine or serum urea concentrations during the weeks following the induction of chronic renal failure. The details of this are listed in Table 3. A recent investigation (53) has demonstrated that there is no apparent limitation to the compensatory growth. Kaufman et al. (54) reported that the increase in the renal mass correlates directly with the amount of tissue excised. Compensatory changes result in a 50% increase following 75% nephrectomy. Shimamura and Morrison (55) commented that the partially nephrectomized rats remain functionally compensated for months before renal failure sets in. During this process, glomerular sclerosis gradually develops and leads to renal decompensation. Shea and associates (53) indicated that the glomerular hypertrophy in this system may itself be pathologic and foreshadow the glomerulosclerosis it precedes.

TABLE 3
Compensatory renal hypertrophy and improvement on renal function.

INVESTIGATOR	DURATION OF STUDY	INDEXES OF RENAL FUNCTION		
		Creatinine	BUN	UREA
		mg/dl	mg/dl	mg/dl
Ellis et al. (48)	4 weeks	1.16	89	--
	8 weeks	0.99	70	--
	12 weeks	0.71	53	--
Letteri et al. (49)	4 weeks	1.1	--	19.2
	8 weeks	0.96	--	130.0
	12 weeks	0.69	--	144.0
Anagnostou et al. (51)	1 week	--	200	--
	2 weeks	--	160	--
	3 weeks	--	120	--

Models with partially ablated renal mass and/or irradiation.

Considerable efforts have been made by investigators to overcome renal hypertrophy in animal models of renal insufficiency. As early as 1905, Passler and Heinecke (56) removed small portions of the remaining hypertrophied tissue in sub-totally nephrectomized dogs. They emphasized that the effect of partial nephrectomy on hypertension associated with cardiac hypertrophy, persisted long after the hypertrophied stumps had been removed. In an attempt to investigate controlling factors in compensatory hypertrophy, Goldman (57) fasted or fasted-refed young adult male rats with unilateral nephrectomy. He found that total fasting after nephrectomy does block compensatory hypertrophy.

Schmid et al. (58) reported that the resection of the hypertrophied portions of the residual kidney in uremic rats allowed long term demonstration of increased brain histidine content which was directly related to the severity of azotemia, a condition comparable to that reported in uremic patients. Mehls et al. (59) were able to reduce the creatinine clearance to 20% of that of control animals by combining seven-eights nephrectomy with local irradiation of 100 rad. Sterner and associates (35) reviewed the work on the reduction of hypertrophy in experimental chronic uremic rats with five-sixths nephrectomy. After the resection of the upper and lower poles of the left renal parenchyma, the remaining tissue was irradiated with 290 rad on each side, while the rest of the abdominal organs were protected by a lead shield. Bleeding was minimized during the resection of the kidney by placing a clamp on the kidney vessels. This process allowed the production of a more profound uremia, with a creatinine clearance of 10% of controls. The rat models were hyperkalemic,

hypocalcemic, hyperphosphatemic, anemic, and slightly acidotic; abnormalities which compare very well with those found in patients with chronic renal failure. Heuck and co-workers (60) were able to produce uremic rat models by a two-stage eight-tenths nephrectomy: sub-total nephrectomy of one kidney with irradiation of the parenchyma with 1,000 rad followed by resection of the contralateral kidney after eight days. These rats demonstrated increased serum concentration of all lipid classes: triglycerides, phospholipids, total and free cholesterol.

Models with partially ablated renal mass and/or electrocoagulation. Several investigators (36,61,62) claim that the use of irradiation to minimize hypertrophy is not only expensive and less readily available, but a method that may produce side effects of nausea and lack of appetite which may aggravate the uremic condition under study. This concern has caused researchers to turn to other approaches, such as electrocoagulation. In this effort, Kampstrup et al. (63) made rabbits uremic by puncturing the renal parenchyma 100 times with an electrically heated needle causing extensive cauterization, except for the upper and lower poles and the hilus. This resulted in a blood urea concentration between 60-180 mg/dl, serum creatinine of 2.2-5.5 mg/dl and serum triglyceride level of 80-178 mg/dl. They claimed this approach demonstrated that a cholesterol diet is not the cause of hypertriglyceridemia that appears in uremic rabbit models.

Combining five-sixths nephrectomy with the application of thermocoagulation on the cut surfaces and the surface of the remnant kidney tissue, Ylitalo et al. (61) were able to exhibit that reduction of the renal mass itself (in adult male rats) favors an increase in blood-pressure

with sodium being only an additional factor. Lim and her associates (62) noted that the resection of the upper and lower poles of the left kidney of adult male rats and cauterization of the raw surfaces, prevents blood loss and minimizes compensatory hypertrophy. They acknowledged that this animal model may be very important in the study of the changes in the thyroid hormone profile in the circulation of chronic renal failure.

Grimmel et al. (64) were able to produce a stable chronic renal failure in male Wistar rats without any hypertrophy. Forty-two days after the removal of the right kidney and the resection of both poles of the left kidney, they made another partial resection of the residual kidney with a thermocautery. This approach resulted in an elevated BUN level (69.0 $\pm$ 2.6 mg/dl in uremic rats and 22.3 $\pm$ 2.0 mg/dl in controls).

Boudet and colleagues (36) also described a method of producing stable chronic uremia by electrocauterization. Adult male Wistar rats were made uremic by inserting a specially devised 16-needle probe into the whole surface of the left kidney. Each needle was 1 mm long and 0.59 mm thick; separated by 1.2 mm. Electrocautery was carried out with a coagulator. Current intensity and length of contact were established to avoid tissue cauterization. The kidney was replaced and the abdominal wall was closed. Seven days later, a contralateral nephrectomy was performed after ligation at the hilus. General laparotomy was done in both sham and experimental animals. As compared to most techniques for inducing chronic renal failure, post operative mortality was nil in this method. Experimental animals developed chronic renal failure which remained stable for months, with a creatinine clearance about 80% less than that in controls. They claimed that hemostasis can be established by superficial

cortical electrocoagulation, a method which avoids the necessity to use various anti-hemorrhagic procedures (28). This method neither requires subsequent destruction of regenerating renal tissue (58) nor preventive irradiation (35).

Immunological Approach

Currently, attempts are being made to understand the mechanisms of immunologic glomerular injury through the use of animal models with reference to the effect of this injury on functional alterations of the disesed kidney. Immunologic interactions are considered to be the major cause of glomerular disease in humans requiring kidney transplants (65).

Models with anti-GBM (glomerular basement membrane disease).

Early work done in this area has incriminated antigen-antibody interaction as a major cause of renal glomerular disease in man. Extensive pathological studies both in man and experimental animal models have revealed that the structure of the glomerular basement membrane is the site of the main pathological changes and the site of fixation of the anti-GBM-antibodies (66).

Wagnild and Gutman (67) developed a dog model of chronic glomerulonephritis limited to one kidney by infusing sheep nephrotoxic serum (NTS) directly into one renal artery, while clamping the pedicle of the protected kidney for as long as 15 minutes. Data obtained in this study suggest that an "autoimmune" hypothesis or a lymphocyte-bound factor is not important in sustaining nephrotoxic nephritis (NTN).

Kondo and associates (68) intravenously injected large amounts of nephrotoxic duck antibody in adult albino rabbits and observed that glome-

rular changes are characterized by a marked increase of monocytic cells with prominent disorganization of the glomerular structure, leading to disappearance of some lobili, with cells migrating into the Bowman's capsular space through the destroyed GBM. Clinical features include massive proteinuria, hyperalbuminaemia, hypercholesterolaemia, disturbances of water and salt excretion, generalized oedema without disturbances in urea, and creatinine excretion. This led to the suggestion that epithelial proliferation is intimately related to glomerular injuries.

Lubec (66) injected rats with rabbit-anti-rat-GBM-antiserum into the tail vein and observed that this cause Masugi type (allergic pathogenesis of nephrotoxic nephritis) of glomerulonephritis in the heterologous phase similar to that in man. This investigator concluded that collagenolytic system is involved in the pathogenesis of the disease. That this collagenolytic process is achieved by degrading the structural associated collagen of the glomerular basement membrane. Couser and co-workers (69) described studies of experimental autoimmune glomerulonephritis (EAG) in guinea pigs infused with GBM prepared from normal human kidney; they indicated that the glomerular injury is mediated by anti-GBM-antibody through complement independent mechanisms.

In an attempt to study whether indomethacin has any beneficial effect on the synthesis of GBM, Daha et al. (70) induced NTN in rats by injecting them with kidney-fixing antibodies (KFAb) and instituted daily administration of the drug at 1,6, or 21 days. They observed the NTN in two seperate phases. A heterologous phase occurred as a result of fixation of KFAb to the antigenic sites present on the GBM, marked by proteinuria; and an autologous phase occurred after day seven when autologous

antibodies, combined with the heterologous protein in the GBM. Data collected indicated that indomethacin has more direct effect on the processes which mediate increased permeability of the GBM, a condition which acknowledged the use of the drug in patients with chronic glomerulonephritis on a trial basis.

Models with immune complex disease. In an attempt to establish the circumstances in which the immune complexes are formed during the course of immune complex disease, Flueren and colleagues (32) intravenously injected Wistar rats with an excess of rabbit anti-rat-brush border (FxIA) antigen or (FxIA) antibody. The results of this study indicated that heterologous immune complex glomerulonephritis is induced during a state of antibody excess.

Allison and her associates (33) used micropuncture, clearance, immunofluorescence and light microscopy techniques to study kidney structure and single nephron function in rats with autologous immune complex nephritis (AICN). Wistar rats were given rear foot pad injections of partially purified rat renal tubular antigen (RTA) in complete Freund's adjuvant containing Mycobacterium butyricum. Observations in the AICN rats suggested that immunopathology can induce tubular injury, (a condition often seen in patients with immune complex glomerulonephritis, particularly when nearing end-stage).

Klassen et al. (71) showed that rabbits immunized with homologous renal tissue developed glomerulonephritis, characterized by deposition of immunoglobulin and complement components along the basement membrane of the proximal tubules and by the development of interstitial fibrosis with minimal irregular infilteration of mononuclear cells. This demonstrates that when there is injury to the GBM, antigen may leak out of the tubular

cells and react with antibody to form the deposits. Salant and others (34) studied the mechanisms of immune deposit formation in the passive Heyman nephritis (PHN) in rats, a model that closely resembles membranous nephropathy in man. PHN was induced by a singe intravenous injection of sheep antibody to rat proximal tubular brush border antigen. Data suggested that deposits may form locally by binding free antibodies to a fixed glomerular antigen rather than circulating complex trapping. Careful sequential studies of the development of this lesion between antibody injection and appearance of proteinuria, revealed that complement was a prominent early constituent of the subepithelial immune deposits, independent of neutrophils.

Salant et al. (62) established a model of membranous immune complex nephritis (passive Heyman nephritis) by a single intravenous injection of ^{125}I-labelled sheep immunoglobulin antibody to rat tubular brush border antigen. They observed that the kinetics of deposit formation in immune complex disease differ markedly from those in antiglomerular basement membrane disease and suggested that factors in addition to antigen-antibody interaction are involved in determining this unique pattern of immune deposit formation.

Couser et al. (63) studied the effect of increased capillary permeability on glomerular immune complex localization in rats immunized with proximal tubular antigen (FxIA) to induce autologous immune complex nephropathy (AICN). Granular deposition of IgG and FxIA were observed within the glomerular mesangium in AICN rats, demonstrating that properties of the glomerulus itself significantly affect the site and quantity of complex deposition.

Questions could be asked as to the validity of experimental glomerulonephritis in animal models, since this disease is usually inferred as the nephrotic syndrome. The renal insufficiency seen in humans may be attributed to damage to any part of the kidney. Surgical reduction of the nephron mass may lead to glomerular obsolescent formation (55). Therefore, the immunological models are of particular interest, if a proper basis for more definite prognosis and maintenance of renal disease is to be achieved.

Chemical Approach

There is abundant documentation that certain chemicals in the environment or drugs commonly used for diagnostic and therapeutic purposes may pose very real risks of nephrotoxicity (74-76). Several investigators attempted to reduce these risks by producing uremic animal models with chemical agents and elucidating the precise mechanisms.

Models with chronic renal failure (CRF) induced by analgesic agents. Though the major role of analgesic agents are documented to add to or to potentiate the toxicity of other analgesics (74), sodium salicylate given to dogs (doses sufficient to produce plasma concentrations comparable to those in human toxicity) caused increased renal losses of water, sodium, potassium, chloride, phosphate and glucose (77). Aspirin by itself, given in moderate doses over a long period (200 mg/kg/day for 66 weeks) under conditions of dehydration, resulted in papillary necrosis in rats (78). When either phenacetin or acetomenophen (1.25 mg/ml) were given alone, very high doses of 500 mg/kg/day were required to cause toxicity (79).

Models with CRF produced by antimicrobial drugs. Among the microbial drugs, aminoglycoside doses equivalent to therapeutic doses in humans is widely known to cause hematuria, oliguria, azotemia, and proteinuria in adult Sprague-Dawley rats (80). The explanation was that there was an involvement of an allergic mechanism marked by eosinophillia. Single intravenous administration (10 mg/100 g rat weight) of the aminonucleoside of puromycin, (6-dimethylamino-9, 3-amino-3'-deoxy-β-D-ribofuranosyl-purine) produced a nephrotic syndrome marked by proteinuria, hypercholesterolemia, hypoproteinemia and edema in rats (81). This model is very similar to human nephrotic syndrome caused by various disease states.

Models with CRF elicited by heavy metals. Current evidence suggests that lead has toxic effect on proximal tubular epithelial cells (31) and releases tubular epithelial protein. The circulating protein is perceived as an antigen, against which antibody is produced, and results in circulating antigen-antibody complexes which result in glomerular damage mediated through the complement system (69).

Models with CRF produced by miscellanous agents. Olessen et al. (82) suggested that long-term lithium administration in adult rats may lead to focal interstitial fibrosis and nephron atrophy. Potassium has a general preventive effect against this lithium induced pathophysiological changes of the kidney.

Intraperitoneal injections of alpha-amanitin toxin in doses of 300 mg/10ml/kg in adult male Wistar rats, induced renal damage characterized physiologically by serious depression of glomerular filteration rate from 8.82 $\pm$ 2.33 ml/min/kg to 2 73 $\pm$ 1.40 ml/min/kg, and a rise in plasma urea

concentration from 51.78 ±17.4 mg/dl to 252.65 ± 94.30 mg/dl (83).

Periodic intravenous injection (three times in 12 days) of uranyl nitrate of 2.0 mg/kg in adult female mongrel dogs, caused a rise in plasma urea nitrogen concentration (from 18.7 ± 3.3 - 290 mg/dl), a decrease in hematocrit without severe anemia, a fall in urine volume to less than 100 ml/dl and an abrupt depression in mean creatinine clearance from 63.6 ± 13.0 - 10.2 ± 2.7 ml/min ($P < 0.001$). The dogs developed proteinuria, aminoaciduria weight loss, lack of appetite, listlessness, lithargy, and plasma amino acid levels similar to those of chronically uremic humans and rats. Repeated injections of uranyl nitrate appeared to produce a reliable chemical approach for creating chronic renal failure (30).

MATERIAL AND METHODS

Care of Animals

Adult male rats[1] weighing 200-250 g, were housed individually in stainless steel cages under constant environmental conditions (24 ± 1°C and 40-42% relative humidity) with a 12 hour photoperiod. Animals were maintained on a commercial stock diet[2] and water ad libitum. Animals were divided into two trials, (trial 1 and trial 2). Animals in trial 1 consisted of 2/3 sub-total nephrectomized rats and sham operated controls. Animals in trial 2 were divided into 2/3 sub-total nephrectomized, sham operated controls and unoperated controls. Trial 1 was designed primarily as pilot project to learn surgical techniques and evaluate the animal response to surgery.

Surgery

Sub-Total Nephrectomy

Animals were sedated by intraperitoneal injection of Nembutal sodium[3] (2.0 mg/100 g body weight). The fur was shaved on the right side from the bottom of the rib cage to the hind limb and scrubbed with Betadine[R] skin cleanser[4]. The area was draped and a 2.0-2.5 cm lateral incision was

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[1]Sprague-Dawley rats, Gibco Animal Resource Laboratory, Madison, Wisconsin, 53701.

[2]Laboratory Rat Chow formulated by the Department of Grain Science and Industry, Kansas State University, Manhattan, Kansas, 66506. (This chow supplies all the nutrients and energy requirements for the laboratory rat as established by the National Research Council, National Academy of Sciences).

[3]Nembutal sodium (Sodium Pentobarbital Injection) Abbott Laboratories, North Chicago, Ill. 60064.

[4]Betadine[R] skin cleanser (Povidone-iodine) Purdue Frederick Company, Norwalk, Conn. 06856.

made (1.0 cm) below the rib cage directly above the kidney. The kidney was identified and carefully lifted from the body cavity (by adjoining fatty tissue) which was subsequently teased away with forceps. The capsule of the kidney was carefully removed, preserving the adrenal glands from injury. With the aid of a Zeiss surgical microscope[1] the three branches of the renal artery were identified. The two anterior branches of the renal artey were carefully disected from adjoining connective tissue and ligated with Ethicon[2] cardiovascular silk suture with tapared surgical needle. Successful ligation resulted in ischemic infarction of 1/2 to 2/3 of the renal mass. Blanching of the infarcted renal mass was evident within 30 seconds of ligation. The damaged kidney was returned in situ and the wound closed with Vetafil Bengon[R] white suture thread, size 00. Following the surgical procedure the rat was loosely wrapped in a light cloth to help maintain body temperature.

Seven days after vascular ligation of the right kidney, the rat was lightly anesthesized with ether and a left nephrectomy was performed vide supra.

Sham Operation

Sham operated control rats were sedated with Nembutal sodium (vide ante) and both right and left kidneys decapsulated during single surgical procedure.

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[1]Carl Zeiss microscope, West Germany.

[2]Ethicon 5-0 cardiovascular silk thread with attached RB-1 tapered S. Jackson Inc. Washington D.C. 20014.

[3]Vetafil Bengon[R] white suture thread, size 00. S. Jackson Inc. Washington D.C. 20014.

Blood Collection

Seven, 14,21, 28, and 35 days following surgery blood was collected from the tail vein for blood urea nitrogen (BUN), serum creatinine, and hematocrit determinations.

Sacrifice

Thirty-five days after surgery rats were fasted for twelve hours and given either an oral glucose load of 350 mg per 100 g body weight (Group A) or water (Group B) via a three-inch curved intubation needle[1*]. After a two hour waiting period, rats were sacrificed by cardiac puncture. Blood samples were centrifuged in a clinical centrifuge at 4°C for 15 minutes. Serum was decanted and stored at -20°C for later analysis.

Analytical Techniques

Blood urea nitrogen was determined by the urease method (84) as published in Clinical Laboratory Methods (85). Serum creatinine was done by the Jaffe-reaction as published in Fundamentals of Clinical Chemistry (86). Total cholesterol was determined by the method of Parekh and Jung (87) as published in Clinical Laboratory Methods (85). High density lipoproteins (HDL) were separated from the serum by a modification of the methods of Lopez-Virella et al. (88). HDL extract was subjected to cholesterol determination as described under total serum cholesterol analysis. Serum triglyceride was done according to the method of Fletcher (89) published in Clinical Laboratory Methods (85).

[1*]Intubation needle, Popper and Sons, Inc. New Hyde Park, New York. 11040. The glucose solution was given with the intension of determining lipogenic enzymes in the liver. However, due to problems with laboratory methodology the results were invalid.

Statistical Analysis

All data collected from the various measurements were subjected to analysis of variance, and the means were separated by LSD with $P = 0.10$, 0.05, 0.01, and 0.001 as appropriate when the F-test rejected the hypothesis of equal means.

RESULTS

Body Weight Change

Trial 1 (35 days). The effect of partial nephrectomy on weight change is presented in Table 4. Thirty-five days after surgery nephrectomized rats weighed significantly less ($P < 0.01$) than sham-operated animals of comparable age. Uremic rats lost an average of 38 g during the study, whereas the sham operated controls lost an average of 25 g.

Trial 2 (35 days). Final body weights of the sham operated and the nephrectomized rats were not significantly different (Table 4). Nephrectomized rats gained substantially less weight (18.5 $\pm$ 10.9 g) than either sham operated (74.8 $\pm$ 13 g) or the unoperated rats (53.4 $\pm$ 15 g). Weight change in nephrectomized rats was not uniform. Some rats demonstrated a gain in body weight, while others showed a loss in body weight, One rat in particular, lost 101 g in the study.

Hematocrit

Trial 1. Table 5 illustrates changes in hematocrit in trial 1 and and 2. The mean hematocrit of nephrectomized rats following surgery was lower (47.1%) than that of the sham operated animals (51.8%) ($P < 0.01$). Hematocrit in both nephrectomized and sham operated controls continued to decline throughout the study (nephrectomized, 42.9%) and (sham controls, 46.5%). However, the hematocrit values in the two groups were not significantly different at the end of the study. Three moderately to severe uremic rats with a blood urea nitrogen (BUN) of 50-71 mg/dl had hematocrit values between 20-25% at the end of the study.

TABLE 4
Average body weight change at seven and thirty-five days following surgery.

VARIABLE	Trial 1			Trial 2		
	Body Weight[1]			Body Weight[2]		
	Initial 7days	Final 35 days	Average Weight Change	Initial 7 days	Final 35 days	Average Weight Change
	g	g	g	g	g	g
2/3 NX[3]	433.8 [a] ±16.7 (n=22)	396.0 [a] ±16.8	-37.8 [a] ±4.7	424.6 [a] ±12.9 (n=13)	443.1 [a] ±13.1	18.5 [b] ±10.9
Sham Operated	435.1 [a] ±21.8 (n=13)	410.2 [a] ±21.9	-24.9 [b] ±6.1	407.8 [a] ±15.4 (n=9)	482.5 [ab] ±15.8	74.8 [a] ±13.1
Unoperated Controls	--	--	--	445.8 [a] (n=7)	499.2 [b]	53.4 [a]

Values represent the mean ± standard error.

[1]Values in the same column with different superscripts are significantly different at the $P < 0.01$ level.

[2]Values in the same column with different superscripts are significantly different at the $P < 0.05$ level.

[3]NX - Nephrectomized rats.

TABLE 5
Hematocrit values at seven and thirty-five days after surgery.

VARIABLE [1]	Trial 1		Trial 2	
	HEMATOCRIT[2]		HEMATOCRIT[3]	
	Initial 7 days	Final 35 days	Initial 7 days	Final 35 days
	%	%	%	%
2/3 Nephrectomized	47.1 [a] ±0.7 (n=22)	42.9 [a] ±1.8	46.5 [b] ±0.8 (n=13)	44.5 [b] ±1.5
Sham Operated Controls	51.8 [b] ±0.8 (n=13)	46.5 [a] ±3.3	48.8 [ab] ±0.9 (n=9)	52.5 [a] ±1.7
Unoperated Controls	--	--	50.7 [a] ±1.1 (n=7)	52.5 [a] ±2.0

[1] Values represent the mean ± standard error.

[2] Values in the same column with different superscripts are significantly different at the $P < 0.01$ level

[3] Significant at the $P < 0.05$ level.

Trial 2. While hematocrit values in sham control rats rose from 48.8% to 52.5%, and in unoperated controls, from 50.7% to 52.5%, hematocrit values decreased in the nephrectomized rats from 46.5% to 44.5%. (Table 5). The hematocrit value of one rat (which lost 101 g of weight) decreased from 44% to 25% with obvious clinical symptoms of anemia (general weakness, skin palor, and loss of appetite).

Blood Urea Nitrogen (BUN)

Trial 1. BUN determinations were conducted at 7,14,21,28 days after surgery and on the day (35 days) of sacrifice. Mean concentrations of urea nitrogen in blood are shown in Table 6. Nephrectomized rats demonstrated a moderate uremia, with a BUN value of 34.8 $\pm$1.74 mg/dl. The BUN of the nephrectomized rats was significantly ($P < 0.01$) higher than that of sham operated controls (24.5 $\pm$ 1.6 mg/dl).

Trial 2. Blood urea nitrogen concentrations were determined at 7, 14,21,28 days after the induction of chronic renal failure, and on the day (35 days) of sacrifice as in trial 1. As expected, the BUN was significantly higher in the nephrectomized rats ($P < 0.001$) than in either sham operated rats or the unoperated controls (Table 6). BUN in sham operated rats was not significantly different from the unoperated controls at 7,21, and 28 days measurements. The BUN in the unoperated controls was significantly lower than the sham controls ($P < 0,001$) on day 14 and 35. In both trial 1 and 2 2/3 nephrectomized rats demonstrated a significantly increased BUN at each time period compared to sham operated controls (trial 1 and 2) and unoperated controls (trial 2).

TABLE 6

Blood urea nitrogen values 7,14,21,28, and 35 days after surgery.

VARIABLE	BLOOD UREA NITROGEN[1]									
	TRIAL 1					TRIAL 2				
	7 days	14 days	21 days	28 days	35 days	7 days	14 days	21 days	28 days	35 days
	mg/dl					mg/dl				
2/3 NX[2]	38.3^{a} ±2.0	36.2^{a} ±2.6	36.3^{a} ±2.6 (n=22)	33.8^{a} ±2.1	34.8^{a} ±1.7	32.4^{a} ±2.8	38.1^{a} ±5.4	42.7^{a} ±5.3 (n=13)	42.9^{a} ±6.3	33.6^{a} ±3.0
Sham Operated Controls	27.9^{b} ±2.6	25.1^{b} ±1.01	24.6^{b} ±0.8 (n=13)	25.7^{b} ±0.9	24.5^{b} ±1.6	12.8^{b} ±3.4	15.4^{b} ±0.6	19.2^{b} ±0.4 (n=9)	17.3^{b} ±0.6	15.8^{b} ±0.7
Unoperated Controls	--	--	--	--	--	11.4^{b} ±0.9	12.5^{c} ±0.7	19.2^{b} ±0.51 (n=7)	15.9^{b} ±0.7	12.8^{c} ±0.8

[1]Values in the same column with different superscripts are significantly different at the $P < 0.001$ level.

[2]NX - Nephrectomized rats.

Serum Creatinine

Trial 1 and 2. No significant difference existed in the serum creatinine level among nephrectomized rats and sham operated controls in trial one (Table 7). Statistically, the nephrectomized rats in trial 2, had a slight increase in the serum creatinine level (0.46 mg/dl) as compared to sham operated controls (0.34 mg/dl). However, they were not different from unoperated controls (0.42 mg/dl). These changes were probably not of significance physiologically.

Total Serum Cholesterol

Total serum cholesterol concentrations in Trial 1 and Trial 2 (Table 8) were significantly higher ($P < 0.01$) in the nephrectomized animals than in the sham operated control rats. In trial 2, the level of the total cholesterol in the sham and the unoperated controls was not significantly different.

High Density Lipoprotein (HDL)

Trial 2. Table 9 shows that HDL concentration tended to be significantly lower ($P < 0.01$) in both the nephrectomized and sham operated rats than the unoperated rats (97.1 $\pm$5.4 mg/dl). HDL levels in nephrectomized rats (70.4 $\pm$ 6.5 mg/dl) and sham controls, (71.8 $\pm$ 7.4 mg/dl) was not different.

Serum Triglycerides

Trial 2. No significant difference existed in serum triglycerides between the nephrectomized rats, sham operated rats or unoperated control rats (Table 9).

TABLE 7

Serum creatinine values at thirty-five days after surgery.

VARIABLE[1]	Trial 1	Trial 2
	Creatinine[2]	Creatinine[2]
	mg/dl	mg/dl
2/3 Nephrecto-mized	0.340 [a] ±0.02 (n=22)	0.46 [a] ±0.02 (n=13)
Sham Operated Controls	0.346 [a] ±0.02 (n=13)	0.34 [b] ±0.02 (n=9)
Unoperated Controls	--	0.42 [ab] ±0.02 (n=7)

[1]Values represent the mean ± standard error.

[2]Values in the same column with different superscripts are significantly different at $P < 0.05$ level.

TABLE 8

Serum total cholesterol at 35 days after surgery.

VARIABLE[1]	TRIAL 1 Total Cholesterol[2]	Trial 2 Total Cholesterol[3]
	mg/dl	mg/dl
2/3 Nephrectomized	109.60±5.5[a] (n=22)	120.5±6.2[a] (n=13)
Sham Operated Controls	91.40±2.6[b] (n=13)	91.0±7.5[b] (n=9)
Unoperated Controls	--	88.5±8.5[b] (n=7)

[1]Values represent the mean ± standard error.

[2]Values in the same column with different superscript are significantly different at the $P < 0.05$ level.

[3]Values in the same column with different superscript are significantly different at the $P < 0.01$ level.

TABLE 9

Serum HDL cholesterol and serum triglycerides at thirty-five days after surgery.

VARIABLE[1]	Trial 2	
	HDL CHOLESTEROL	SERUM TRIGLYCERIDES
	mg/dl	mg/dl
2/3 Nephrectomized[2]	70.4[a] ±6.5	62.3[a] ±9.8
Sham Operated[3] Controls	71.8[a] ±7.4	59.2[a] ±7.1
Unoperated[4] Controls	97.1[b] ±5.4	49.8[a] ±8.6

[1]Values represent the mean ± standard error.

[2]n=13

[3]n=9

[4]n=7

Values in the same column with different superscripts are significantly different at $P < 0.01$ level.

DISCUSSION

Data has been presented on the induction of kidney insufficiency in the rat by ligation of selected renal arteries. The change in body weight, low hematocrit level, high BUN, high total cholesterol, and low high-density lipoprotein values indicated that a mild to moderate uremic model had been successfully produced.

The present study indicated that moderate uremia may impair growth rate in the rat. Holliday (2) has made a similar observation in chronic uremic children. Letteri et al. (49) described retarded weight gain in uremic rats and explained that skeletal defect may simply be a manisfestation of the overall depression of body growth in animals ill with uremia. The mechanisms by which uremia affects the growth rate are unknown. Chantler and his associates (4) attributed this defective growth rate to increased caloric requirements. While Wang and co-workers (8) documented that protein is utilized less efficiently in uremic rats. Kopple and Swenseid (90) proposed that there may be impairment of protein synthesis related to a decreased availability of certain amino acids, or to an abnormal hormonal activity. Alternatively, Felig et al. (91) inferred that protein degradation may be enhanced in uremia.

Surgical manipulative sham procedures and the induction of uremia in this study seem to have an effect on hematocrit concentration. Similar findings were observed by Schultz et al. (50). The mechanism of anemia in renal insufficiency is still obscure. Most of the studies have demonstrated that anemia of uremia is due to hemolysis from shortened red blood cells survival, secondary to uremic toxins and occasionally aggravated

by hypersplenism (92) or due to decreased production of renal erythropoietic stimulating factor (ESF) as a result of insufficient erythropoietin (50). Others believe that there may be a blockage in the further differentiation into morphologically recognizable erythroid precursor cells (93).

The decreased hematocrit found in the nephrectomized and sham operated animals in this study is in part related to the blood loss at the time of surgery and the routine blood sampling. However, the significant differences in hematocrit values between sham operated and nephrectomized rats (trial 2) 35 days after surgery suggest that the anemia may be due to those mechanisms which may be operating as a consequence of the uremia.

The finding of two to three fold elevation of blood urea nitrogen (BUN) levels in the rats with remnant kidney left after partial nephrectomy shows that mild to moderate uremia has been produced. This result compares well with that of the work on rats by Bagdade et al. (43), Soman and Felig (44), and with those found in patients with chronic renal failure (45). The accumulation of urea in the blood is due to decreased excretory function of the diseased kidney. Urea, a diamide of carbonic acid, manufactured soley in the liver as a chief end-product of protein catabolism, is documented as the major nitrogenous constituent of the urine. Hence, the excretion of urea has been used to reflect glomerular function. Therefore, any diminution in glomerular clearance, whether isolated or due to an organic lesion or to any functional upset, is reflected in diminished out put of urea by the kidney.

Most investigators rely on urea, creatinine or inulin clearances as a yardstick of kidney function. However, whether these clearances have the same significance in uremic patients as in normal individuals, is not fully known. Results of many investigators (94) have demonstrated that the rate of production of end-products of nitrogen catabolism is constant and they are completely excreted by the kidney. Therefore, changes in glomerular filteration rate can be inferred from changes in the blood levels of these substances.

The low serum creatinine concentrations observed in the sub-totally nephrectomized rats in both trial 1 and 2 are in aggreement with the findings of Ormrod and Miller (28) who documented substantially low levels of serum creatinine in moderate and mild uremic rat models. This finding of low creatinine levels in uremic models suggests that chronic renal failure in the rat may ensue long before any disturbances of creatinine clearance may be observed. To explain this phenomenon, Huttunen et al (35) proposed that factors such as bodily wasting, trauma, and fever due to infection may reflect the rate at which creatine of muscle mass is produced. Though some of the nephrectomized rats in this study failed to gain weight, there was no evidence of bodily wasting, or any environmental circumstances causing trauma. The low levels of serum creatinine observed in the sub-totally nephrectomized rats of this study suggested that the kidney dysfunction was moderate to mild. However, other investigators (35,36,44) have observed an increase serum creatinine as a result of experimentally induced renal damage.

In contrast to the usual finding in uremic patients, serum total cholesterol was constantly elevated in the nephrectomized rat models in

this study. This result is in accordance with Heuck et al (60) who found high serum cholesterol values in uremic rat models. Too, Kamstrup et al. (63) were able to demonstrate in uremic rabbit models that high serum cholesterol values observed in animal models were not due to a high cholesterol diet, but a condition that is attributed to the uremic state itself. The nephrectomized rats in this laboratory, received a laboratory rat chow (formulated by Department of Grain Science and Industry, Kansas State University) which contains negligible amounts of cholesterol. Nevertheless, results from human studies (21) suggested that total cholesterol levels are usually normal or low in patients with chronic kidney failure. Norbeck et al. (95) demonstrated in uremic patients with hyperlipidemia that the serum total cholesterol content was about 82% below the median values for their control group. In contrast, Okubo and his associates (96) found elevated serum cholesterol levels in post-transplant patients.

The decreased high density lipoprotein concentration observed in the sub-totally nephrectomized rats in this study has been frequently observed in chronic renal failure. Bagdade and Albers (97) have recently reported that HDL concentration is subnormal in both dialyzed and nondialyzed uremic patients. Savdie et al. (98) also reported that HDL is reduced in concentration and altered in composition in chronic renal failure in rats. In a prospective evaluation by Savdie and other co-workers (99) the behaviour of HDL concentrations after grafting was found to depend on renal function.

The mechanism underlying reduced HDL concentrations in chronic renal failure have not been elucidated. Epidermiological studies have

linked low concentrations of HDL with an increased risk of coronary heart disease (100). Because of this reciprocal relation between low levels of HDL and the prevalence of ischemic heart disease, the measurement of HDL has recently acquired considerable importance. Glomset (101) indicated that the effect of HDL may be related to its action in removing free cholesterol from peripheral cells and transporting it to the liver. Too, Carew et al. (102) inferred that HDL may also regulate the uptake of low-density lipoprotein by the tissue. Rapoport and associates (103) found abnormal HDL composition in uremic patients with hyperlipidemia. They proposed that in patients on chronic hemodialysis, the uptake of cholesterol from peripheral tissue is impaired due to the abnormal HDL. Levy et al. (104) acknowledged that HDL is a carrier of the apolipoproteins necessary for the lipolytic breakdown of very-low-density-lipoprotein to low-density-lipoprotein. So a low level or abnormal composition of HDL may be of primary importance for the raised levels of lipoproteins, and for the development of atheromas in vessel walls.

The concentration of serum triglycerides in the nephrectomized rats in this laboratory was lower than other findings (43). In fact, both human and animal experimental studies (1,43) have documented that elevated triglycerides are a well recognized accompaniment of chronic uremia. Bagdade et al. (43) postulated that the removal of triglyceride rich lipoprotein from the circulation, may be reduced due to functional impairment of the kidney and increased hepatic triglyceride production. Nevertheless, most triglyceride studies in uremic animal models are based on high fat, and high carbohydrate diets, since this is the dietary

regimen of patients with chronic kidney dysfunction (24).

Data from this study indicate that the surgical procedure used in this laboratory can result in a rat with evidence of impaired renal function.

SUMMARY

A model is described for the induction of uremia in the rat by ligation of two-thirds of the renal arteries. 'Mild' to 'moderate' uremia was produced by a combination of unilateral nephrectomy of the right kidney and selective ligation of the primary and secondary divisions of the left renal arteries in adult male Sprague-Dawley rats. This approach reduced the functional mass of the left kidney by one-half to two-thirds.

A sham operative single procedure of decapsulating the two kidneys provided appropriate control for the model. The two-stage procedure resulted in elevation of urea nitrogen two to three-fold over control rats. These uremic rat models demonstrated decreased weight gain (18.5 g in experimental compared to 74.8 g, sham or 54.4 g, normal control), decreased hematocrit concentration (44.5%, experimental vs 52.5%, control), two fold elevated total serum cholesterol value over controls, and sub-normal level of HDL cholesterol value. Since these are characteristic conditions associated with the uremic state in animal models, the surgical procedure used in this laboratory can result in a rat with evidence of impaired renal function.

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APPENDIX

TABLE 10A

Initial body weight, final body weight, total weight change; Initial and final hematocrit (HCT) of individual animals.

FIRST TRIAL 2/3 NEPHRECTOMIZED GROUP

	BODY WEIGHT			HEMATOCRIT (HCT)	
Rat	Initial	Final	Weight Change	Initial	Final
	g	g	g	%	%
1	296	259	-37	52	38
2	314	265	-49	44	25
3	293	281	-12	45	45
4	298	242	-56	48	41
5	305	293	-12	48	48
6	399	317	-82	46	47
7	289	265	-24	47	49
8	286	251	-35	46	45
9	526	505	-21	42	48
10	537	460	-77	43	25
11	509	499	-10	48	50
12	542	537	- 5	43	48
13	568	526	-42	45	44
14	520	438	-82	48	20
15	529	473	-56	46	53
16	529	488	-41	43	46
17	516	494	-22	52	45
18	477	458	-19	48	47
19	450	414	-36	49	43
20	405	388	-17	54	45
21	450	416	-34	50	44
22	507	444	-63	49	47
Mean ± SEM	434 ± 17	396 ± 17	-38 ± 5	47 ± 0.7	43 ± 2

TABLE 10A (Continued)

FIRST TRIAL SHAM OPERATED GROUP

Rat	BODY WEIGHT			HEMATOCRIT (HCT)	
	Initial	Final	Weight Change	Initial	Final
	g	g	g	%	%
1	410	408	- 2	47	55
2	433	424	- 9	53	46
3	434	424	-10	52	49
4	447	418	-29	46	51
5	411	404	- 7	47	56
6	452	400	-52	45	52
7	477	462	-15	50	55
8	414	357	-57	45	54
9	403	377	-26	45	53
10	420	383	-37	43	52
11	454	434	-20	48	50
12	465	432	-33	43	51
13	438	415	-23	43	50
Mean ± SEM	435 ± 22	410 ± 22	-25 ± 6	46 ± 3.3	52 ± 0.8

TABLE 11A

Initial body weight, final body weight, total weight change; Initial and final hematocrit (HCT) of individual animals.

SECOND TRIAL 2/3 NEPHRECTEMIZED GROUP

Rat	BODY WEIGHT			HEMATOCRIT (HCT)	
	Initial	Final	Weight Change	Initial	Final
	g	g	g	%	%
1	386	430	+44	47	49
2	431	510	+79	48	48
3	456	430	-26	40	38
4	371	379	+ 8	41	39
5	403	486	+83	49	48
6	451	502	+51	47	49
7	420	382	-38	46	40
8	404	411	+ 7	51	45
9	440	444	+ 4	50	50
10	470	369	-101	44	26
11	355	411	+56	48	50
12	438	508	+70	44	52
13	499	477	-22	50	42
Mean ± SEM	423 ± 13	443 ± 13	+18 ± 11	46 ± 0.8	44 ± 1

TABLE 11A (Continued)

SECOND TRIAL SHAM OPERATED GROUP

Rat	BODY WEIGHT			HEMATOCRIT (HCT)	
	Initial	Final	Weight Change	Initial	Final
	g	g	g	%	%
1	320	467	+147	48	53
2	420	529	+109	46	52
3	453	523	+ 70	44	59
4	424	490	+ 66	48	48
5	457	520	+ 63	51	54
6	440	512	+ 72	52	51
7	468	552	+ 84	49	53
8	367	414	+ 47	49	50
9	336	371	+ 35	51	54
Mean ± SEM	408 ± 15.4	482 ± 16	75 ± 13	49 ± 0.9	52 ± 1.7

NORMAL CONTROL GROUP

1	461	525	+ 64	52	53
2	402	422	+ 20	50	55
3	410	500	+ 90	50	53
4	425	473	+ 48	51	52
5	461	515	+ 54	49	50
6	460	518	+ 58	52	53
7	499	539	+ 40	51	52
Mean ± SEM	446 ± 17	499 ± 18	+ 53 ± 15	51 ± 1.1	52 ± 2.0

TABLE 12A

Blood urea nitrogen (BUN), serum creatinine, serum total cholesterol of individual animals.

FIRST TRIAL 2/3 NEPHRECTOMIZED GROUP

Rat	BLOOD UREA NITROGEN 7 days	14 days	21 days	28 days	35 days	Serum Creatinine	Total Cholosterol
						mg/dl	mg/dl
1	56.4	37.9	39.3	33.5	30.6	0.40	92.9
2	41.1	45.0	50.0	45.7	43.6	0.36	70.6
3	42.5	32.0	27.1	24.4	25.9	0.32	114.1
4	28.2	27.9	33.6	22.0	22.9	0.26	97.8
5	41.1	45.7	40.0	27.4	36.0	0.26	108.8
6	28.9	34.3	43.6	26.7	36.7	0.44	98.4
7	37.5	38.6	37.1	37.3	37.3	0.24	99.5
8	30.7	25.7	32.1	25.9	28.1	0.26	76.1
9	32.9	32.1	30.7	32.0	29.8	0.28	81.5
10	50.7	52.0	50.0	49.5	55.6	0.36	108.7
11	26.4	21.4	22.9	25.9	25.4	0.26	81.5
12	30.0	35.0	32.9	29.7	33.1	0.38	157.6
13	30.0	37.1	30.0	31.2	30.9	0.22	76.1
14	65.7	66.4	71.4	59.4	48.1	0.44	98.8
15	31.4	35.0	27.1	23.6	33.4	0.36	125.0
16	42.8	64.3	45.7	44.2	34.2	0.40	135.9
17	26.1	24.3	24.3	27.4	32.2	0.38	108.7
18	50.7	22.1	39.3	34.3	44.0	0.42	119.6
19	40.7	33.6	34.3	41.9	41.5	0.52	157.6
20	30.7	32.8	34.3	38.9	37.9	0.34	146.7
21	47.9	18.6	25.7	34.3	30.7	0.28	114.1
22	30.0	33.6	26.4	28.2	27.1	0.32	141.3
Mean	38.3	36.2	36.3	33.8	34.8	0.34	109.6

TABLE 12A (Continued)

FIRST TRIAL SHAM OPERATED GROUP

Rat	BLOOD UREA NITROGEN 7 days	14 days	21 days	28 days	35 days	Serum Creatinine	Total Cholesterol
						mg/dl	mg/dl
1	43.5	22.1	30.0	27.4	29.0	0.38	103.3
2	25.7	30.0	21.4	22.1	24.0	0.32	76.1
3	28.6	27.0	29.3	29.7	38.6	0.64	76.1
4	25.7	24.0	24.3	25.9	22.9	0.30	86.9
5	22.9	24.0	22.1	28.9	16.4	0.38	86.9
6	31.4	23.6	25.0	25.1	16.1	0.18	103.3
7	25.0	24.0	24.3	23.6	22.2	0.30	97.8
8	29.3	21.0	25.7	32.0	26.2	0.28	92.4
9	25.7	21.0	24.3	25.1	21.9	0.34	86.9
10	25.7	31.0	24.3	25.1	25.9	0.36	86.9
11	30.0	30.0	25.7	22.9	28.3	0.28	86.9
12	25.7	22.0	22.1	23.6	20.4	0.36	103.3
13	22.9	27.0	21.4	22.7	26.0	0.40	97.8
Mean	27.9	25.1	24.6	25.7	24.5	0.35	91.4

TABLE 13A

Blood urea nitrogen (BUN), serum creatinine, serum total cholesterol, HDL cholesterol, and triglycerides of individual animals.

SECOND TRIAL 2/3 NEPHRECTOMIZED GROUP

	BLOOD UREA NITROGEN						Serum Cholesterol		
Rat	7 days	14 days	21 days	28 days	35 days	Creatinine	Total	HDL	Triglyceride
						mg/dl	mg/dl	mg/dl	mg/dl
1	27.0	22.5	25.0	27.0	26.0	0.34	118.7	87.7	66.1
2	26.2	30.0	35.0	26.1	29.2	0.34	112.5	75.1	22.3
3	42.2	57.0	59	67.3	41.9	0.60	168.8	38.7	47.3
4	44.1	61.5	60.0	68.0	43.7	0.50	162.5	84.7	72.0
5	27.2	21.0	30.0	27.7	26.8	0.38	112.5	75.1	37.6
6	25.3	30.0	35.0	31.8	26.8	0.40	93.8	96.8	37.6
7	34.0	36.0	37.0	39.6	27.6	0.54	100.0	50.8	45.2
8	34.0	36.0	50.0	42.8	41.8	0.46	118.7	128.9	64.5
9	22.5	25.5	31.0	28.6	27.6	0.36	118.7	106.5	71.5
10	74.1	87.0	96.0	100.3	62.4	0.76	112.5	92.0	110.8
11	21.5	28.5	31.0	27.9	30.8	0.56	106.3	93.2	59.7
12	25.3	22.5	31.0	24.3	23.7	0.32	81.3	50.8	35.5
13	21.5	37.5	35.0	47.2	29.2	0.52	156.3	101.1	99.5
Mean	32.4	38.1	42.7	42.9	33.6	0.46	120.2	70.4	62.3

TABLE 13A (Continued)

SECOND TRIAL SHAM OPERATED GROUP

Rat	BLOOD UREA NITROGEN 7 days	14 days	21 days	28 days	35 days	Creatinine	Serum Cholesterol Total	HDL	Triglyceride
						mg/dl			mg/dl
1	16.4	19.5	21.0	19.0	20.5	0.32	106.3	104.1	44.1
2	11.2	18.0	20.0	14.9	18.1	0.36	93.8	89.0	60.2
3	13.5	16.5	20.0	18.5	14.2	0.32	87.5	69.0	54.8
4	12.2	12.0	18.0	17.4	17.3	0.28	112.5	68.4	81.7
5	12.5	18.0	19.0	16.7	14.0	0.34	75.0	76.3	40.9
6	13.1	15.0	17.0	14.2	14.9	0.34	81.3	56.9	37.1
7	14.1	15.0	20.0	18.8	14.2	0.40	106.3	97.4	105.4
8	10.3	12.0	19.0	18.5	12.6	0.38	78.1	46.6	25.3
9	12.7	15.0	20.0	18.1	16.6	0.34	81.3	42.4	10.6
Mean	12.8	15.4	19.2	17.3	15.8	0.34	91.0	71.8	59.2

NORMAL CONTROL GROUP

1	13.1	13.5	17.0	15.3	14.2	0.52	100.0	92.0	30.1
2	11.2	10.5	21.0	15.8	11.8	0.38	81.3	77.5	36.0
3	9.5	10.5	18.0	15.3	11.4	0.46	75.0	62.3	23.1
4	15.0	13.5	19.0	17.4	13.9	0.44	68.8	145.9	75.8
5	12.6	16.5	21.0	14.2	14.4	0.38	81.3	142.2	75.3
6	8.9	10.5	18.0	14.4	11.8	0.40	100.0	74.4	98.4
7	9.0	12.0	20.0	19.7	11.4	0.44	118.8	124.1	76.3
Mean	11.4	12.5	19.2	16.0	12.8	0.42	88.5	97.1	49.8

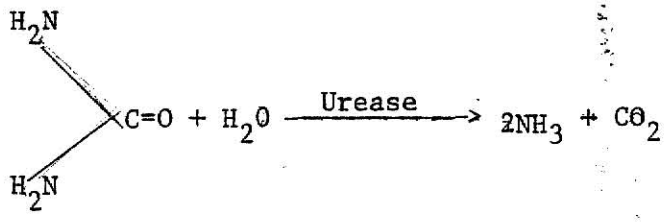
ANALYTICAL PROCEDURES

Blood urea nitrogen

The serum urea nitrogen was determined by the highly sensitive, simple and rapid procedure of a modified urease-Berthelot reaction (84)

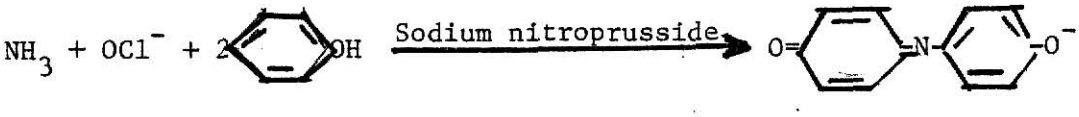
Principle:

1. Urea is hydrolyzed by urease to ammonia and Co_2.



Urea Ammonia

2. Ammonia then reacts with alkaline hypochlorite and phenol in the presence of a catalyst, sodium nitroprusside ($Na_2Fe(CN)_5NO \cdot 2H_2O$), to form indophenol. The concentration of ammonia is directly proportional to the absorbance of indophenol, which is measured spectrophotometrically at 570 nm.



Phenol Indophenol (Blue)

Reagents

Alkaline hypochlorite: Dissolve 12.5 gm sodium hydroxide in about 400 ml water. Cool, add 20 ml sodium hypochlorite solution (any commercial bleach solution containing 5.25% available chlorine), and dilute to 500 ml. Store in a polyethylene bottle in the refrigerator.

Phenol nitroprusside solution: Dissolve 25 gm phenol and 0.13 gm sodium nitroprusside in water to make 500 ml. Store in a brown bottle in the refrigerator. This remains stable for several months.

Buffer: Dissolve 5 gm disodium salt of EDTA in 200 ml glycerin and 250 ml water. Adjust to pH 6.5 with 4% sodium hydroxide (about 10 ml required) and dilute to 500 ml.

Buffered urease: Dissolved 30 mg urease type III (Sigma) in 100 ml buffer. This remains stable for several weeks when stored in the refrigerator.

Benzoic acid, 0.016 M: Dissolve 2 gm benzoic acid in one liter water. Add 0.8 ml concentrated sulfuric acid and mix.

Stock standard: Dissolve 0.644 gm urea in some of benzoic acid (0.016 M) solution and dilute to 500 ml. This standard contains 60 mg/dl urea nitrogen.

Working standard: Make a 1:2 dilution of the stock standard to give 30 mg/dl standard.

Procedure. To an aliquot of 0.5 ml buffered urease, add 0.02 ml serum. This solution was incubated for 15 minutes at 37^{o}C to hydrolyze urea into ammonia. 1.0 ml of phenol nitroprusside reagent was added and the solution was well mixed. Another 1.0 ml of alkaline hypochlorite reagent was also added and mixed. 5 ml of ammonia-free water was added and the tube was

allowed to develop color at room temperature for 20 minutes. The solution was read spectrophotometrically against a reagent blank at 570 nm.

Calculation:

$$\text{BUN (mg/dl)} = \frac{\text{absorbance of test}}{\text{absorbance of standard}} \times 30$$

Serum creatinine determination

Serum creatinine was determined by the Jaffe-reaction as published in Fundamentals of Clinical Chemistry (86).

Principle: Creatinine is determined in a protein-free filterate serum after applying the Jaffe reaction. This results in the production of an amber colored substance of unestablished composition after addition of an alkaline picrate solution.

Reagents:

1. Sulfuric acid, 2/3 (0.66) N: Slowly add 18.8 ml of concentrated H_2SO_4 to about 500 ml of water. When cool, dilute to 1 liter.
2. Sodium tungstate, 10% (0.3) M: Dissolve 50 g reagent grade sodium tungstate ($Na_2WO_4 \cdot 2H_2O$) in distilled water and dilute to 500 ml.
3. Sodium hydroxide, 0.1 N: Dissolve 4 g of NaOH in H_2O to one liter.
4. Sodium hydroxide, 10%: Dissolve 40 g of sodium hydroxide in distilled water to make one liter. Store in polyethelene or alkali-resistant bottle.

5. Picric acid, 0.04 M: Dissolve about 9.3 g of picric acid in about 500 ml of water at 80°C. Cool to room temperature, dilute to one liter with distilled water and titrate with 0.1 N NaOH using phenolphthakein as the indicator. Dilute as necessary to make 0.04 M.

6. Creatinine standard:

   A. Stock standard: Dissolve 2 mg pure creatinine and one ml concentrated hydrochloric acid (HCL) in distilled water to make 100 ml. This solution contains 2 mg/ml and is stable when kept in refrigerator.

   B. Working standard: Dilute 0.2 ml of stock standard (1 mg/1 ml) in 100 ml distilled water. Working standard=2 mg/dl. This is because serum is diluted 1:10 when test is run but the standard is not.

7. Alkaline picrate: Mix together 5 volumes of picric acid to 1 volume of 10% NaOH. This solution must be made up immediately before use.

Procedure: A 1.0 ml of serum was pipetted into a large tube and 7.0 ml distilled water was added to dilute the serum. 1.0 ml of 2/3 N H_2SO_4 was added, followed by 1.0 ml of 10% $Na_2WO_4 \cdot 2H_2O$ to precipitate the proteins. The test tube was stopped and shaked vigorously and the solution was centrifuged at 1700 rpm for 5 minutes. 5.0 ml of the filtrate was added into a cuvette and 2.5 ml alkaline picrate was added. The solution was allowed to stand at room temperature for 20 minutes for color development. The tube was read against a reagent blank at 520 nm spectrophotometrically.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 2 = \text{mg/dl creatinine}$$

Total serum cholesterol

Total serum cholesterol was determined by the method of Parekh and Jung (92) as published in Clinical Laboratory Methods (85).

Reagents:

1. Ferric acetate-uranyl acetate solution (FA-UA): 0.5 g $FeCl_3.6H_2O$ in 10 ml of water in a centrifuge tube. Add 3 ml of concentrated ammonium hydroxide. Centrifuge, decant and discard supernatant and wash the precipitate three times with water. Dissolve the precipitate in 1 L of acetic acid, add 0.1 g powdered uranyl acetate and mix well. Allow the solution to stand overnight then mix well. The solution is stable for 6 months in a brown bottle.
2. Sulfuric acid-ferrous sulfate solution (SA-FS): Dissolve 0.1 g anhydrous ferrous sulfate in 100 ml glacial acetic acid in a 1 L volumetric flask. Add 100 ml concentrated sulfuric acid while swirling. After cooling to room temperature, dilute to volume with sulfuric acid.
3. Cholesterol standard: Dissolve 250 mg pure cholesterol in chloroform to make 100 ml.

Procedure: A 50 ul portion of serum was added to 10 ml of FA-UA solution to precipitate the proteins and extract the cholesterol. The solution was allowed to stand 5 minutes, then centrifuged at 1,700 rpm for 5 minutes. A 3 ml aliquot of the supernatant was transferred to another tube, 2 ml of SA-FS solution was added slowly down the side of the tube. The solution was mixed well and allowed to stand for 20 minutes for color development. The tubes were read against a blank of 3 ml FA-UA and 2 ml SA-FS at 560 nm with

a Bausb & Lomb Spectronic 20 spectrophotometer.

Calculation:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{Conc. of standard}$$

= mg cholesterol/dl.

Serum triglycerides

Serum triglycerides were determined by a modification of the method of Fletcher (89) as published in Clinical Laboratory Methods (85).

Principle:

Triglycerides are extracted from serum with isopropanol. Interfering substances, including glucose, glycerol, phosphatides and bilirubin, are removed by a solid absorbant. The triglyceride-containing extract is then subjected to the following reaction:

1. Triglycerides + KOH $\longrightarrow$ glycerol + fatty acids
2. Glycerol + periodate $\longrightarrow$ formaldehyde
3. Formaldehyde + NH_4^+ + acetylacetone diacetyl dihydrolutidine (a yellow color exhibiting maximum absorption at 410 nm.

Reagents:

1. Zeolite mixture: Grind zeolite in a Waring blender for several minutes then heat at 110°C overnight. Mix 100 g zeolite with 10 g calcium hydroxide, 10 g hydrated aluminu- silicate (also called Kaolin or Lloyd's reagent), and 5 g copper sulfate pentahydrate.

2. Potassium hydroxide, 5% (w/v): Dissolve 5 g potassium hydroxide in

isopropanol/water solution (40:60, v/v) to make 100 ml.

3. Sodium metaperiodate, 0.025 M.

   A. Stock solution: Dissolve 5.347 g sodium metaperiodate in 1 L of 1 N acetic acid (58 ml/L water).

   B. Working solution: Prepare fresh daily by diluting 12 ml stock solution and 20 ml isopropanol to 100 ml N acetic acid.

4. Acetyacetone: Dissolve 0.75 ml acetylacetone and 2.5 ml isopropanol in 100 ml of 2 M ammonium acetate (154.2 g/L water). The solution is stable for one month in a brown bottle in the refrigerator.

5. Triolein standard: Dissolve 120 mg of triolein in isopropanol to make 100 ml. The solution is stable in the refrigerator.

Procedure.

Extraction. A 0.1 ml aliquot of serum or standard was added to 4.9 ml isopropanol. One g of zeolite mixture was added to the serum, standard or blank (5 ml isopropanol) and mixed on a vortex mixer for 20 seconds. The tubes were centrifuged at 2800 rpm for 5 minutes. The supernatant was decanted into another set of tubes and centrifuged.

Saponification: A 2 ml aliquot of the supernatant was transferred to capped tubes and 0.6 ml of 5% potassium hydroxide solution was added. The tubes were mixed by swirling, capped and incubated for 15 minutes in a 60^{o}C water bath for 15 minutes for color development. After cooling to room temperature the tubes were read at 410 nm spectrophotometrically.

Calculation:

$$\frac{\text{Absorbance of test}}{\text{Absorbance of std.}} \times 300 = \text{Triglycerides (mg/dl)}$$

High-density lipoprotein cholesterol determination

High density lipoproteins were separated from the serum by modification of the method of Lopez-Virella et al.(88).

Reagents:

1. Sodium phosphotungstate solution: Dissolve 40 g of phosphotungstic acid per liter of a solution of 1 M NaOH and distilled water (16/84, v/v).

2. $MgCl_2$ (2M).

Procedure:

To 0.5 ml of fresh serum, 0.075 ml of phosphotungstate solution was added, mixed, and 0.0125 ml of $MgCl_2$ was added and mixed well. The tubes were allowed to stand for 5 minutes, then centrifuged at 2500 rpm for 10 minutes and 5000 rpm for 30 minutes. The supernatant was analyzed for cholesterol as described under total cholesterol.

Calculation:

$$\frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{concentration of standard} \times 1.175$$

= mg HDL cholesterol/dl.

A SURGICAL RAT MODEL FOR CHRONIC RENAL FAILURE

by

OKAILEY ARYEE

B.S. (MT), Rush Medical College, Chicago, Ill., 1976

AN ABSTRACT OF A MASTER'S THESIS

submitted in partial fulfillment of the

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MASTER OF SCIENCE

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KANSAS STATE UNIVERSITY
Manhattan, Kansas

1982

ABSTRACT

A model is described for the induction of uremia in the rat by ligation of two-thirds of the renal arteries. 'Mild' to 'moderate' uremia was produced by a combination of unilateral nephrectomy of the right kidney and selective ligation of the primary and secondary divisions of the left renal arteries in adult male Sprague-Dawley rats. This approach reduced the functional mass of the left kidney by one-half to two-thirds.

A sham operative single procedure of decapsulating the two kidneys provided appropriate control for the model. The two-stage procedure resulted in elevation of urea nitrogen two to three-fold over control rats. These uremic rat models demonstrated decreased weight gain (18.5 g in experimental compared to 74.8 g, sham or 54.4 g, unoperated control), decreased hematocrit concentration (44.5%, experimental vs 52.5%, control), two-fold elevated total serum cholesterol value over controls, and sub-normal level of HDL cholesterol value. Since these are characteristic conditions associated with the uremic state in animal models, the surgical procedure used in this laboratory can result in a rat with evidence of impaired renal function.