AN ABSTRACT OF THE THESIS OF

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Title: The Effect of Dietary Protein Level on Plasma Urea and Transaminase Activities in Uremic Rats

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The effect of dietary protein level on plasma urea nitrogen, glutamic-oxalacetic (GOT) and glutamic-pyruvic (GPT) transaminase activities was studied in uremic rats.

Uremic, sham-operated and unoperated female rats weighing approximately 150-250 g were matched according to body weight and assigned at random to diets containing 5% protein (casein) and 23% protein (Purina Lab Chow).

At both levels of protein intake uremic rats gained less weight than their controls, and the sham-operated rats. The urea concentration was higher in both groups of uremic rats, being significantly higher in the group fed 23% protein, while both GOT and GPT enzyme activities were dramatically lower in comparison with the sham-operated and the unoperated control rats. However, the levels of the transaminase enzymes were lower in the uremic rats fed 23% protein, in which the plasma urea concentration was higher than in the uremic rats fed 5% protein. The higher transaminase

activities, along with lower urea levels in the 5% protein-than the 23% protein-fed rats indicates that a reduced protein intake may be of benefit in reducing the severity of the toxic symptoms associated with renal insufficiency.

These data indicate that time, diet and uremia induction have direct relationships with body weight gains and urea plasma concentrations. Inversely proportional relationships were observed for GOT and GPT activities with respect to both diet and time. The low activities of both GOT and GPT found in the uremic rats may be due to an inhibition by urea or by some other substances present in the uremic serum. The reduction of the transaminase enzymes to near zero activities in the chronically uremic rats suggests a possible importance of these enzymes in the clinical diagnosis of renal disorders in man.

# THE EFFECT OF DIETARY PROTEIN LEVEL ON PLASMA UREA AND TRANSAMINASE ACTIVITIES IN UREMIC RATS

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# INTRODUCTION

Uremia is a toxic condition associated with renal insufficiency and the retention of nitrogen substances in the blood which are normally excreted by the normal functioning kidney (Tabor, 1970). The clinical syndrome of uremia in chronic renal failure is a composite of complex abnormalities which eventually affects virtually all tissues and organs. In its most extreme form uremia may induce mental deterioration, coma, convulsions, muscle weakness and wasting, pericarditis, cardiac arrhythmia, anemia, bleeding tendencies, severe loss of appetite, vomiting and weight loss (protein and fat).

Many theories have been advanced to explain the toxic symptoms which characterize uremia. Harrison and Mason (1937) reviewed the early literature on the pathogenesis of uremia. They reported that azotemia (high urea blood levels) was an early manifestation of uremia. However, it soon became apparent that although urea itself could induce uremic symptoms, other nitrogenous substances, such as creatinine, were equally effective. Because of the varied symptoms associated with the uremic syndrome, it was early suggested that a multitude of toxic agents were involved in the production of uremia.

Both organic and inorganic imbalances have been conjectured to be associated with many of the clinical symptoms of uremia (Harrison and Mason, 1937). Edmund (1927) showed that the complete clinical, histological, and chemical symptoms of uremia could be produced by injecting hypertonic salt solutions into dogs, in which urine production was suppressed by acidosis. Sharp, et. al. (1964) studied primates which were trained on a simple avoidance schedule during the following

three experimental uremic states: 1) continuous urine infusion, 2) bilateral ureteral ligation, and 3) bilateral nephrectomy. Performance decrements occurred under the latter two conditions when blood urea nitrogen (BUN) concentrations reached 195 mg %, and both were directly correlated with plasma potassium or sodium ion concentration. Harrison and Mason (1937) have suggested that organic anions, such as oxalate and citrate, may contribute significantly to the regulation of blood ions levels because of their chelating abilities. In a preliminary study Villamil (unpublished) observed that dietary protein may significantly alter electrolyte balance, since there was a decrease in urinary volume in uremic rats fed 5% protein (casein). A small increase in the urinary magnesium concentration also was observed, but a decrease was found for chloride, sodium, potassium and calcium in contrast with the control group fed 5% protein. In opposition with the results obtained for the sham-operated controls fed 5% protein, uremic rats demonstrated an increase in urea, chloride, magnesium and calcium. The uremic group fed 23% protein (casein) exhibited an increased urinary volume, and a slight increase in magnesium, but urea, chloride, sodium, potassium and calcium concentrations were decreased in comparison with the controls fed the same diet. Uremic rats fed 23% protein (Purina Lab Chow) had lower urine levels of urea, sodium, chloride, potassium, and magnesium, but greatly increased calcium levels than controls fed the same diet. Mackay, et. al. (1927) observed that in uremic rats there was an hypertrophy of the adrenal glands, due somewhat to an increase in water content of the glands, but in large part to hypertrophy of the cortex. The content of water and substances soluble in fat solvents were higher in uremic rats than in controls.

De Olivera, et. al. (1966) observed that in acute experimental uremia in rats, the development of acute lung changes were prevented by intravenous injection of nephrotic immune serum. Acute uremia caused no structural changes in the lungs that could obviously hinder the contact between circulating antibodies and local antigens. It was suggested that acute uremia interferes with some final mechanism through which the inflammation, secondary to the antibody-tissue-antigen reaction, is brought about. Pasternack (1964) showed that pulmonary changes may develop as part of the uremic syndrome without hyperhydration. Ether and nembutal anesthesia increased the incidence and severity of the pulmonary changes. It is possible that this may be an associated underlying cause of the detriment to the capillaries.

Stanley, et al. (1966) studied the bleeding tendency in uremia and showed that platelet counts and plasma coagulation factors were normal. There was a significantly inverse correlation between the level of serum urea nitrogen and creatinine, and cell-adhesiveness was significantly lower in bleeding subjects than in those without bleeding. These observations suggest a direct effect of urea, or of its metabolites, on platelet function, and that urea may be instrumental in causing the bleeding defect during uremia.

Galloway, et al. (1964) observed that uremic subjects without diabetes, liver and central nervous system disease, or contributing drug therapy had an elevated serum pyruvate correlated with the degree of nitrogen retention. Blood lactate, however, was found to be no different than in the normal control group. Schmidt, et al. (1950) observed that the plasma of uremic patients exhibited markedly elevated levels of conjugated p-creasol (2 to 18 fold). Both the free and the conjugated

aromatic hydroxyacid fractions were greatly elevated in uremia. Since these compounds are relatively non-toxic, they concluded that the symptoms of uremia are apparently not due to the free or conjugated phenolic compounds in the blood.

Biro, et al. (1965) found that under physiological conditions, the tissues concentrations of urea nitrogen, creatinine, and uric acid were uniformly higher than in serum. This situation remained unchanged under uremic conditions for all substances, except urea nitrogen, which showed the following gradient: liver>serum>tissues. It was found that only some of these substances are capable of diffusibility in experimental uremia. The concentration of urea becomes lower during hemodialysis, and it is only after its termination that urea levels in the serum fall below that in the intracellular compartment. Czerniack (1970) showed that the levels of all examined peptide-bound amino acids, except aspartic acid, were significantly changed in uremia. Alanine, cysteine, phenylalanine and glutamic acid levels were significantly decreased, while the ratio of N-substituted to peptide-bound amino acids was elevated. The levels of peptide-bound glycine also were significantly increased. Muting, et al. (1967) observed that severe uremia was found to be associated with highly significant increases in amino acids in the cerebrospinal fluid. In particular, aromatic amino acids and glutamic acid were elevated. This increase probably reflects increased levels of the fee amino acids and increases in the permeability of the blood-brain barrier during uremia.

Mitsuko, et al. (1971) observed that the growth of rats fed 70% casein was retarded one week after vitamin $B_6$ depletion, while rats fed a 10% casein diet showed normal growth during four weeks, regardless

of whether the diet was with or without vitamin $B_6$. Liver transaminase activities in rats on $B_6$-depleted high protein diets were decreased, especially alanine transaminase. Ornithine transaminase activity also was decreased.

Chan, et al. (1974) studied uremic rats fed 11% and 18% protein casein diets and observed that the urea clearance of uremic rats in both groups was reduced below their controls. The low urea clearances in the uremic rats were associated with higher plasma urea levels than the controls. Uremic rats fed 11% protein had lower plasma urea levels than those fed 18% protein.

Daubresse, et al. (1976) studied plasma lipids and lipo-proteins in a group of chronic uremic patients, some of whom were maintained by regular hemodialysis. Compared with healthy individuals there was a significant increase in plasma triglycerides and in the prebeta-1- and prebeta-2-lipoprotein plasma concentrations. There was no difference between dialyzed and undialized uremic patients. Carbohydrate intake was normal, and plasma insulin and free fatty acid levels were within the normal range. There was no correlation between plasma triglyceride levels and the degree of hypoalbumina. Basal plasma glucagon levels were high in nearly all dialyzed patients, and post-heparin lipoprotein lipase activity was very low in dialyzed patients. It was also found that regular hemodialysis for 32 weeks did not improve hypertriglyceridemia.

Kempner (1946), Borst (1948), Bull (1952), Colff (1952) and many other investigators have advocated the use of high caloric and essentially protein-free diets in the treatment of patients with chronic uremia. The high caloric intake did permit some degree of "protein sparing", but positive nitrogen balance was not achieved in chronically-ill patients

with depleted protein stores, until 0.25 to 0.5 g of protein/Kg body weight was added to the diet (Herndon, et al., 1958).

Synderman, et al. (1962) gave $^{15}N$-labelled urea and ammonium chloride to four patients maintained on an inadequate diet, and observed the incorporation of the labelled nitrogen into plasma protein and the hemodialysate of infants, who showed a significant weight gain. Rose, et al. (1965), Bloch (1946), Leifer, et al. (1948), and Rust, et al. (1956) also have found that urea and other nitrogenous compounds can be utilized as a source of nitrogen for the anabolism of protein in rodents.

Holliday (1972) observed that food intake in both uremic children and adults is less than that of their weight-matched controls. While a slightly lower intake of nitrogen and salt per kilogram results, the intake of calories also was reduced. This reduction in caloric intake in uremic children is often associated with poor growth and reduced physical activity.

Rigid reductions in protein intake can produce considerable clinical improvements in patients with moderate or far advanced uremia (Giordano, 1963; Scribner, et al., 1965). Wright, et al. (1970) showed that patients with chronic renal failure treated with low protein diets of differing amino acid contents exhibited a more rapid improvement in the rate of metabolic balance when fed diets containing a higher proportion of essential amino acids. Aoyama, et al. (1971) observed that a choline-deficient diet containing 14% protein caused fatty liver, whereas a 30% casein diet deficient in choline did not. The addition of an amino acid mixture simulating 16% casein (total casein level 30%) to a choline-deficient diet prevented lipid accumulation, while supplementation with

methionine alone resulted in a partial decline of the liver lipid content. Methionine, serine, and glutamic acid may be responsible for the lipoproteic action of the casein diet.

Chatterjee, et al. (1971) showed that growth rate and organ weight increased with an increased protein content of the diet from 0 to 25%, however, those values were slightly lower in 60% casein-fed animals. A correlation was demonstrated between the activities of kidney L-gulonate dehydrogenase and 3-oxoi glucoiate decarboxylase and the growth rates, while the activity of liver dehydroascorbatase was significantly reduced in guinea pigs fed diets containing higher levels of protein. Coles (1927) supported the finding that weight loss and other evidences of wasting are commonly observed in patients with renal failure. Want, et al. (1976) showed that in both uremic and controls rats, plasma concentrations of certain amino acids, primarily non-essential ones, varied inversely with protein intake. With a 5% protein diet, the ratio of the essential amino acids to non-essential amino acids was significantly reduced. These observations indicate that both uremia and reduced protein intake may affect amino acid metabolism in rats with chronic renal failure. They also observed that the uremic animals gained less weight and had lower protein efficiency ratios than controls, regardless of diet. In addition, certain plasma amino acids levels were altered in the uremic animals. Those included tyrosine and tyrosine/phenylalanine ratio, which were decreased, and citrulline, glycine, and methylhistidine which were increased. They also found that urea nitrogen and creatinine concentrations were significantly elevated in uremic animals as compared to pair-fed controls. Likewise, urea and

creatinine clearances were significantly reduced in the uremic rats as compared to controls in both high- and low-protein fed rats.

Chantler (1974) found that uremic rats matched for body weight with control rats consumed significantly fewer calories. Multiple regression analysis of weight gain against age and calorie intake suggested that there may be an increase in the calorie cost of growth in rats with uremia. Moise, et al. (1927) demonstrated that adult rats maintained on a high protein diet (85% casein) acquired glomerular and tubular lesions in the remaining kidney at 90, 120 and 150 days after unilateral nephrectomy. They also observed that animals on the high protein diet had a marked increase in albuminuria.

Bowering, et al. (1970) showed that urinary excretion of urea, uric acid, alpha amino acids, and ammonia varied directly with protein intake, but the percentage contribution of individual compounds to total N excretion differed according to each dietary N level. They also found that high protein and RNA diets produced identical urinary acid excretion, but only RNA increased serum urate levels. Schmidt, et al. (1969) found that caloric intake and body weight of rats kept on a protein-deficient diet were markedly lower when the standard diet had been diluted with glucose instead of starch.

Giordano, et al. (1966) indicated that when dialysis was performed at frequent intervals, a reduction in the amounts of plasma free amino acids was evident in patients on a low protein diet. In the case of high protein intake, it has been demonstrated (Eclozeim, 1966; Synderman, et al., 1966) that there is a marked reduction in all essential, as well as some nonessential, amino acids in blood plasma. Patients with uremia on a low protein diet also showed low levels of amino acids in the urine,

both relatively and absolutely. Giordano, et al. (1966) similarly found a reduction in the urine and/or plasma levels of histidine in uremic subjects. Kopple, et al. (1968) showed that there was a significant decrease in serum urea nitrogen in uremic rats fed a diet of 20% protein in comparison with uremic rats fed 40% protein. They also found that both uremic groups exhibited an increase in plasma, histidine, aspartic acid, glycine, and valine. They further observed that plasma pH decreased significantly in both groups, and that there was no significant difference in plasma pH between the two uremic groups.

Warnock, et al. (1974) has stated that low values for glutamic-oxalacetic transaminase (GOT) activity observed in uremic patients might result from several causes, the simplest being a systematic error in the measurement introduced by the use of an Auto Analyzer. On the other hand, if the low values are not a result of the instrumentation used, and do accurately reflect the GOT activity present in the serum, then the decreased activity may be due to: a) a decreased amount of apoenzyme, b) decreased levels of the cofactor pyridoxal phosphate, or c) the presence of an inhibitor in uremic serum. These authors found that GOT activity measured in the sera of uremic patients was, in fact, significantly lower than that measured in the sera of normal individuals, and that those low values were exaggerated with the Auto Analyzer. They also demonstrated that the relationship between serum creatinine (uremic patients, 40 mg/liter) and serum glutamic-oxalacetic transaminase activity is lower in uremic patients, regardless of the method of assay. Cohen, et al. (1976) observed extremely low serum GOT activity (10 IU) in 6% of 5030 samples; 71% of those occurred in patients with azotemia. Those workers also found that GOT activity was inversely

proportional to the serum urea concentration. GOT activity was decreased in uremia, especially when azotemia was advanced. Glynn, et al. (1970) have reported false elevations of serum GOT (Auto Analyzer) with para-aminosalicylic acid, while Sabath and Finland (1966) have reported artifactual increases induced with erythromycin in colorimetric assays for GOT activity.

Cohen, et al. (1976) considered that prompt increases in serum GOT activity after dialysis suggest that an inhibitor or inhibitors are dialyzable. Furthermore, uremic serum in which GOT activity is absent, does not suppress the GOT activity present in azotemic serum. This phenomenon suggests that the bound inhibitor retains its inhibitory effect, but when unbound it is labile. It is of interest that transketolase activity, which has been shown to be decreased in uremic serum, can likewise, be increased after dialysis (Lonergran, et al., 1968; Wolf, et al., 1973).

Tulpule, et al. (1959) studied the GOT activity of whole blood in experimental animals feed low protein or pyridoxine-deficient diets, and found lowered blood concentrations of GOT. This effect was more marked with the combined deficiency of those two nutrients; nowever, when missing nutrients were added to the diet the enzyme activity returned to normal in two to three weeks. They also observed that the effect of added pyridoxine was often more gradual and of a more stable nature than that of protein.

Since recent evidence has indicated that serum transaminases may be decreased in uremic animals, especially when azotemia is advanced, the present study was designed to further investigate the effects of the development of azotemiz and uremiz on both glutamic-oxalacetic

transaminase and glutamic-pyruvic transaminase (GPT) in rats with normal and insufficient renal function. The long established fact that azotemia is inversely proportional to the level of dietary protein intake, and the fact that serum transaminase activities appear to be regulated by blood urea (or its metabolite) concentration, prompted the present investigation as a model for the effect of developing azotemia on plasma transaminase enzyme levels.

# MATERIALS AND METHODS

## Experimental Design

Female Sprague-Dawley rats weighing 150-250 g were made uremic by ligation of two-thirds to three-fourths of the primary and secondary division of the left renal artery, followed one week later by contralateral nephrectomy (Wang, et al., 1976). Two control groups consisted of a sham-operated group which had only the left renal artery ligated, while unoperated animals made up the other group.

After surgery the rats were housed in individual, bottom-screened cages with controlled temperature (21C). The rats were assigned at random to diets providing either 5% protein (casein), or 23% protein (Purina Lab Chow). The composition (g/100 g) of the 5% protein diet was as follows: solka floc 3.0%; casein 4.5%; sucrose 27.6%; dextrin 47.65%; vitamin mix 2.0%; DL-methionine 0.15% (Ralston Purina Co.).

One week after nephrectomy uremic, sham-operated, and unoperated rats ingesting either the 5% protein or 23% protein diets were matched according to body weight. Five rats in each of the dietary, and control or uremic groups, were later sacrificed at three, six and nine weeks after nephrectomy. The animals were anesthetized with ether, and the blood was withdrawn by cardiac puncture and centrifuged in heparinized tubes.

## Enzyme Assays

Plasma concentrations of glutamic-oxalacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were measured with a dual-beam spectrophotometer (Hitachi Perkin-Elmer, 139 UV-VIS) at 340 nm. Commercial

enzyme preparations of Optimized GOT and GPT were obtained from Boehringer Mannheim Corporation. Plasma urea nitrogen was measured by the BUN urease colorimetric method (Boehringer Mannheim Corporation).

## Statistical Methods

Data were statistically treated by using analysis of variance, Duncan's Multiple range test, and the Paired-Sample "t" test, and are expressed as means $\pm$ standard error (Zar, 1974). The difference between means were considered significant if they exceeded table values for "F", and "t" at the 0.05 level of probability.

# RESULTS

## Weight Changes: Effects of Diet and Uremia

With each diet the uremic rats gained less weight than their weight-matched controls (Table I). The difference in weight gain became statistically significant after nine weeks. Also, uremic rats fed 5% protein gained less weight than uremic rats fed 23% protein. The lowest weight gain was in the uremic rats fed 5% protein. Average food intake was about 14 g/day for animals fed 5% protein and about 12 g/day for animals fed 23% protein.

The results demonstrated that the uremic rats fed 5% protein exhibited only a slight increase in body weight with time, while the rats fed 23% protein showed significantly increased body weight with time (Fig. 1A, 1B). In addition, the gain in body weight of the uremic groups was less than in both the unoperated control and the sham-operated rats.

## Plasma Urea Concentration: Changes With Time

Neither the control nor the sham-operated groups fed the 5% protein diet showed any significant changes in plasma urea concentration during the course of the experiment (Table II), however, the uremic rats fed the same diet experienced a 22% increase in urea concentration by six weeks and a greater than 50% increase by nine weeks (Table II). Although both the control and the sham-operated groups fed the 23% protein diet showed a 40-50% increase in plasma urea at the end of the nine weeks, the uremic rats at three weeks had already demonstrated a 55% greater plasma urea concentration than the controls at the same time period. The plasma urea

Table I. Changes in body weight (g) in weight-matched control, sham-operated and uremic rats fed 5% protein (casein) and 23% protein (Purina Lab Chow).

| Weeks | Group | Rats fed 5% protein | | Rats fed 23% protein | |
|---|---|---|---|---|---|
| | | Initial Weight | Final Weight | Initial Weight | Final Weight |
| 3 | Control | 154.0 ± 4.06 | 175.0 ± 4.49 | 170.5 ± 2.23 | 207.0 ± 0.33 |
| 6 | Control | 165.0 ± 2.98 | 184.3 ± 3.89 | 165.0 ± 2.91 | 203.5 ± 0.52 |
| 9 | Control | 210.3 ± 4.61 | 233.8 ± 0.42 | 193.3 ± 0.55 | 247.8 ± 0.23 |
| 3 | Sham | 165.0 ± 2.44 | 184.3 ± 0.53 | 165.0 ± 1.80 | 194.0 ± 0.65 |
| 6 | Sham | 155.0 ± 3.44 | 170.0 ± 1.49 | 170.0 ± 0.31 | 203.5 ± 0.24 |
| 9 | Sham | 180.0 ± 4.59 | 193.2 ± 0.20 | 192.0 ± 0.98 | 235.3 ± 0.41 |
| 3 | Uremic | 150.0 ± 195 | 159.5 ± 0.06 | 150.0 ± 2.54 | 166.8 ± 0.43 |
| 6 | Uremic | 160.0 ± 3.26 | 166.5 ± 0.60 | 165.0 ± 1.58 | 189.0 ± 0.70 |
| 9 | Uremic | 192.5 ± 2.33 | 203.3 ± 1.18 | 250.0 ± 2.50 | 273.2 ± 3.59 |

All values are means ± SE; N=5 in each group

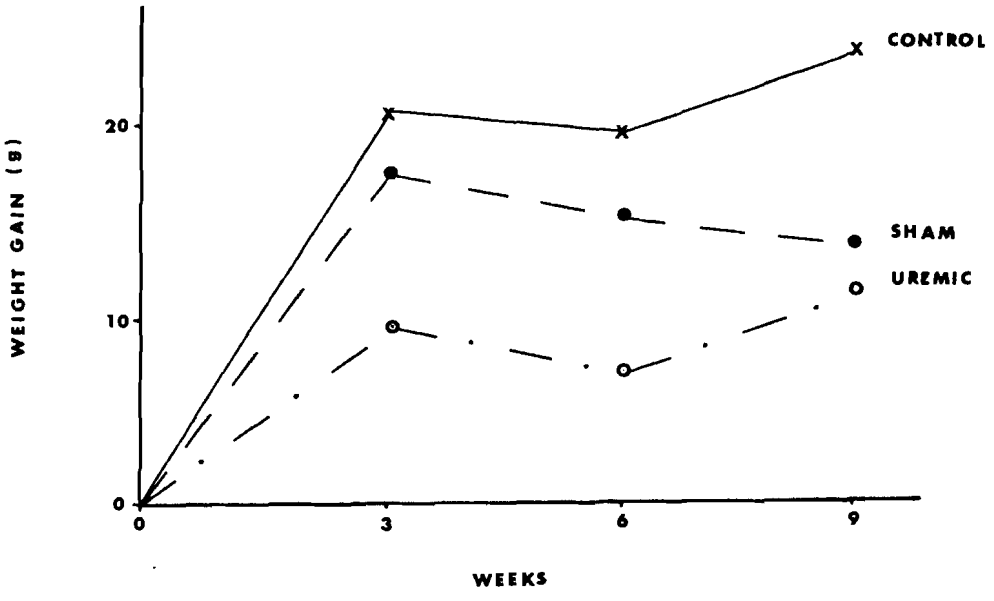


Fig. 1A. Body weight gains in control, sham and uremic rats fed 5% protein diets at three, six, and nine weeks.

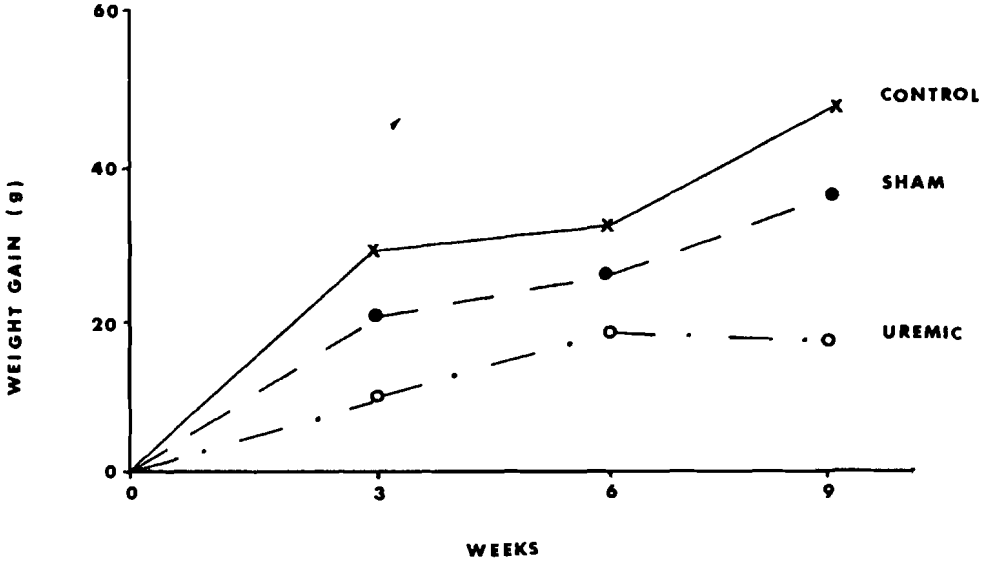


Fig. 1A. Body weight gains in control, sham and uremic rats fed 23% protein diets at three, six, and nine weeks.

Table II. Plasma urea concentration (mg/dl) in control, sham-operated, and uremic rats fed 5% protein and 23% protein for three, six and nine weeks.

| Weeks | Group | Rats fed 5% protein | % of control at same week | Rats fed 23% protein | % of control at same week |
|---|---|---|---|---|---|
| 3 | Control | 10.6 ± 0.80 | ---- | 9.9 ± 0.21 | ---- |
| 6 | Control | 10.1 ± 0.42 | ---- | 12.0 ± 0.48 | ---- |
| 9 | Control | 10.1 ± 2.26 | ---- | 15.2 ± 0.77 | ---- |
| 3 | Sham | 8.8 ± 0.19 | 83.0 | 9.7 ± 0.26 | 98.0 |
| 6 | Sham | 10.7 ± 2.24 | 105.9 | 11.0 ± 0.64 | 91.7 |
| 9 | Sham | 10.3 ± 0.50 | 102.0 | 13.5 ± 1.42 | 88.8 |
| 3 | Uremic | 11.5 ± 0.004 | 108.5 | 15.4 ± 1.09 | 155.6 |
| 6 | Uremic | 14.0 ± 0.71 | 138.6 | 19.5 ± 1.52 | 162.5 |
| 9 | Uremic | 17.3 ± 1.83 | 171.3 | 28.3 ± 1.86 | 186.2 |

All values are means ± SE; N=5 for each group at each time period.

concentration of the uremic rats on the 23% protein diet was dramatically increased over 86% greater than the controls at the end of nine weeks (Table II).

## Plasma Amino Transferases

Changes with Time in Glutamic-Oxalacetic Transaminase (GOT). A comparison of the mean values of GOT activity obtained for the uremic rats fed 5% protein and 23% protein diets showed that the uremic rats fed 5% protein had higher enzyme activities than the uremic rats fed 23% protein diet, except at three weeks (Table III). At nine weeks the control group fed 23% protein exhibited an almost 2.5-fold increase over the controls at three weeks, and a four-fold increase over the nine weeks uremic rats fed 5% protein, however, the enzyme activity of the nine-week uremic rats fed 23% protein was only about 2% of the nine-week control rats fed the same diet. The sham-operated group fed 23% protein demonstrated about the same response as the same rats fed 5% protein, since there were no statistical differences between the rats on either diet at each of the measured time intervals (Table III).

Glutamic-Pyruvic Transaminase (GPT) Changes as a Function of Time. Glutamic-pyruvic transaminase (GPT) activity in the control groups fed 5% protein was high at three weeks, but progressively decreased at six weeks and nine weeks (Table IV). The sham-operated rats fed the 5% protein diet showed no significant differences among these three time periods. The uremic rats fed the same diet showed a markedly reduced GPT activity, which was only about 9-18% of that of the control rats. In general, the rats fed the 23% protein diet showed GPT activities which were lower than the rats fed 5% protein diet (Table IV).

Table III. Glutamic-oxalacetic transaminase activity (mU/ml) in control, sham-operated and uremic rats fed 5% protein and 23% protein for three, six and nine weeks.

| Weeks | Group | Rats fed 5% protein | % of control at same week | Rats fed 23% protein | % of control at same week |
|---|---|---|---|---|---|
| 3 | Control | 26.8 ± 3.61 | ---- | 49.7 ± 4.00 | ---- |
| 6 | Control | 53.0 ± 8.34 | ---- | 42.7 ± 4.47 | ---- |
| 9 | Control | 54.6 ± 7.49 | ---- | 128.9 ± 3.50 | ---- |
| 3 | Sham | 42.6 ± 10.00 | 159.0 | 39.9 ± 6.07 | 80.3 |
| 6 | Sham | 79.3 ± 24.22 | 149.6 | 41.5 ± 3.71 | 97.2 |
| 9 | Sham | 85.6 ± 13.01 | 156.8 | 102.8 ± 26.67 | 79.8 |
| 3 | Uremic | 5.5 ± 0.86 | 20.5 | 13.7 ± 0.87 | 27.6 |
| 6 | Uremic | 28.4 ± 3.30 | 53.6 | 4.4 ± 0.51 | 10.3 |
| 9 | Uremic | 32.2 ± 3.61 | 59.0 | 2.9 ± 0.53 | 2.2 |

All values are means ± SE; N=5 for each group at each time period.

Table IV. Glutamic-Pyruvic transaminase activity (mU/ml) in control, sham, and uremic rats fed 5% protein and 23% protein for three, six, and nine weeks.

| Weeks | Group | Rats fed 5% protein | % of control at same week | Rats fed 23% protein | % of Control at same week |
|---|---|---|---|---|---|
| 3 | Control | 132.3 ± 8.55 | ---- | 60.1 ± 10.15 | ---- |
| 6 | Control | 113.7 ± 11.84 | ---- | 25.7 ± 1.24 | ---- |
| 9 | Control | 85.7 ± 6.27 | ---- | 24.6 ± 3.36 | ---- |
| 3 | Sham | 79.3 ± 9.01 | 60.0 | 31.7 ± 5.77 | 52.7 |
| 6 | Sham | 93.0 ± 16.94 | 81.8 | 66.1 ± 5.14 | 257.2 |
| 9 | Sham | 87.7 ± 18.02 | 102.3 | 87.4 ± 20.53 | 355.3 |
| 3 | Uremic | 11.7 ± 1.02 | 8.8 | 14.8 ± 4.11 | 24.6 |
| 6 | Uremic | 20.5 ± 6.05 | 18.0 | 3.0 ± 0.51 | 11.7 |
| 9 | Uremic | 10.4 ± 2.01 | 12.1 | 2.7 ± 0.43 | 10.9 |

All values are means ± SE; N=5 for each group at each time period.

However, both groups of uremic rats were observed to have strikingly lower GPT activities than both the unoperated control and the sham-operated rats. The uremic rats fed 5% protein had higher plasma GPT activities than the uremic rats fed 23% protein except during the first three weeks when the levels of GPT were not significantly different from that of the uremic rats fed the 5% protein.

# DISCUSSION

## Influences of Diet, Time and Uremia Induction on the Changes in Body Weight

The results of this study demonstrated that changes in body weight were influenced by: a) diet, b) time and c) the uremic condition. The weight gained by uremic rats was less than the non-uremic rats, possibly due to the fact that the uremic rats utilized dietary protein less efficiently than the non-uremic rats. It was also found that the uremic rats fed 5% protein gained less body weight than the uremic rats fed 23% protein (Fig. 1A). This was probably due to the marginal level of protein in the 5% diet, since none of the groups fed that diet showed any significant body weight changes after three weeks. Those results are supported by Wang, et al. (1976), who also showed that uremic rats fed reduced dietary protein grew more slowly, and had altered plasma amino acid levels. Control rats offered the same diets under ad libitum conditions, in contrast, grew rapidly. Uremic rats, at all levels of dietary protein intake, were found to show the lowest increase in body weight. Chantterjee, et al. (1971) showed that growth rate, organ weight and tissue amino acid levels increased when dietary casein was increased from 0 to 25%. However, the tissue amino acid values decreased slightly when the animals were fed a 60% casein diet.

Chantter, et al. (1974) have attributed decreased growth rate in uremic rats to increased caloric requirements. Holliday et al. (1972), who studied the caloric deficiency in children with uremia, observed that children had a reduced tolerance to a low level calorie diet,

while a high level of calories was better tolerated, and produced better growth. It was suggested that there was a relationship between growth and caloric intake, and for some children, an increase in caloric intake by supplementation of the diet was the critical factor which improved growth.

Chan, et al. (1974) observed that body weight increases in uremic rats fed an 18% casein diet were significantly less than controls, even though the food intakes of both groups were similar. They suggested that the deranged metabolism induced by uremia results in an inefficient utilization of nutrients, since the body weight gained for uremic rats fed an 11% protein casein diet, was not significantly less than controls.

## Influences of Diet, Time and Uremia on Plasma Urea Concentrations

The results found in the present study suggest that direct relationships exist among diet, time, and the induction of uremia on plasma urea concentration, since all uremic rats fed either 5% protein or 23% protein diets had higher plasma urea concentrations at each time period than the control, and sham-operated groups fed the same diets (Table II). The maximum plasma urea concentration was found at nine weeks in the uremic rats fed 23% protein. The high level of protein in that diet resulted in a greater nitrogen release from protein breakdown, followed by a concomitant increase in urea formation. Those findings are in agreement with the results obtained by Chan, et al. (1974), who studied uremic rats fed 11% and 18% protein casein diets, and observed that the uremic rats fed 18% casein diets tended to have higher plasma urea values than uremic rats fed 11% casein diet. They also reported

that urea clearance in uremic rats was inversely proportional to plasma urea concentrations at both levels of dietary protein intake when compared with the controls. Villamil (unpublished) has observed that uremic rats fed 23% protein (Purina Lab Chow) had higher urea clearances then uremic rats fed 5% protein (casein).

Kopple, et al. (1968) studied uremic patients who consumed 40 g of a mixed protein diet containing at least one and one-half times the minimal daily requirement of each essential amino acid, or a 20 g protein diet with approximately 14 g of high biological value protein supplied from two whole eggs. They reported that there was a significant decrease in serum urea nitrogen in the group fed 20 g protein, and that the serum urea nitrogen fell the greatest degree in the initial three weeks and tended to stabilize after that.

## Influences of Diet, Time and Uremia Induction on Plasma Transaminase Activities

Influences on Glutamic-Oxalacetic Transaminase. This study found that in both dietary groups of uremic rats the GOT activity was drastically decreased in comparison with the levels obtained for the sham-operated and the unoperated control rats (Table III). Since the uremic rats exhibited the lowest GOT activity at each of the measured time periods, it is very likely that urea or some other substances present in the uremic serum was responsible for the enzyme inhibition. This contention is further supported by the fact that the chronically uremic rats (six and nine weeks) fed 23% protein had GOT activities which were only a modicum of those of the uremic rats fed 5% protein.

These findings were consistent with the results obtained by Wolf, et al. (1972), who found that serum GOT activity was low or absent in uremic patients. Those workers suggested the following possible reasons for this occurrence: 1) the patients had abnormally high serum lactate concentrations, which may have caused a rapid consumption of NADH co-enzyme in the chemical laboratory test, and resulted in an artifactually low GOT; this phenomenon occurs in cases of beriberi, diabetic ketoacidosis, and severe liver disease, or 2) that the enzyme is lost in the course of dialysis. Warnock, et al. (1974) observed that the GOT activity in the serum of uremic patients was low, and suggested that it may be due to: a) decreased amounts of apoenzyme, b) decreased levels of the cofactor pyridoxal phosphate, c) the presence of an inhibitor in uremic serum, or d) some combination of these. They also reported that after myocardial infarction and recurrent severe cardiac episodes, GOT values never exceeded normal limits in uremic patients. In contrast with this finding was the observation that patients with hepatitis exhibited increased GOT activity. Borglin (1958) also found that GOT activity was low or absent in pregnancy and may be associated with low pyridoxine concentrations.

Cohen, et al. (1976) observed that there was low or absent GOT activity in azotemic patients, and also that GOT activity was increased after hemodialysis. When uremic serum in which GOT activity was absent, was added to non-azotemic serum with GOT activity, GOT activity was not suppressed. This phenomenon suggests that when bound in some way, the inhibitor retains its inhibitory effect, but that when unbound, it is labile.

Thus, it seems likely that the decrease in GOT activity found in uremic rats in the present study are probably a result of similar effects,

as mentioned above. Further investigation will be required in order to ascertain the exact inhibitor(s) of GOT activity in uremic animals.

Influences on Glutamic-Pyruvic Transaminase. The results found in this study showed that GPT activity was decreased in both groups of uremic rats in comparison with the levels obtained for the sham-operated and unoperated control rats (Table IV). In both groups of control rats, whether fed 5% protein or 23% protein, a time-dependent decrease in GPT activity was found, which is consistent with the results obtained by Chen, et al. (1975). The increased activity of plasma GPT in the sham-operated group fed 23% protein at six and nine weeks may be due to the tissue-specific release of GPT from the kidney damaged by ligation of the renal artery branches. The greater decrease in GPT activity in the 23% than the 5% protein fed rats could be due to feed-back inhibition from urea (or other metabolite), or to the lower level of the cofactor pyridoxine present in that diet (4 mg/Kg diet). Chen, et al. (1975) studied rats which ingested only 10 to 80$\mu$g of dietary pyridoxine/100 g body weight/day, and showed that the levels of the apoenzyme of GPT in erythrocytes, plasma, and liver were not dependent on the level of dietary pyridoxine. They found, however, that GPT activities in all samples were stimulated by in vitro incubation with pyridoxal phosphate. Therefore, they suggested that the coenzyme pyridoxal phosphate, not the apoenzyme, appeared to be the limiting factor for GPT activity, and that apoenzyme biosynthesis was in excess of the amount of pyridoxal phosphate available, in vivo, at all levels of dietary pyridoxine tested. They also found that GPT activities in the plasma and erythrocytes reached a maximum at a dietary pyridoxine

level of 4 mg/Kg, and that erythrocytes were more sensitive than plasma or liver to vitamin $B_6$ depletion as measured by the parameter of GPT activity.

Correlation of Plasma Transaminases and Urea

The present data demonstrated that when urea plasma levels were high in uremic rats, the plasma transaminase activities were low, meaning that an inversely proportional relationship was found between these two parameters. Those findings are supported by Cohen, et al. (1976), who observed an extremely low serum GOT activity (10 IU) in 331 of 5030 uremic patients (6.5%). In 86 of those subjects, no detectable GOT activity was found, and 85 of these 86 patients had serum urea nitrogen values above 25 mg/dl. Serum urea nitrogen levels were inversely related to serum GOT activity when GOT activities were abnormally low. As the GOT activity increased, both the percentage of patients with azotemia, and the mean serum urea nitrogen concentrations progressively decreased. They observed that GOT activity was decreased in uremia, especially when azotemia was advanced.

Clinical Implications. The syndrome of uremia affects all tissues and organs in the body, and produces a multitude of effects on animals, such as, mental deterioration, coma, convulsions, anemia, vomiting, muscle weakness, altered amino acid metabolism, retarded growth, wasting, cardiac arrythmias, etc. In this study, renal insufficiency produced dramatic effects on plasma urea concentrations, weight gains, and transaminase activities (GOT and GPT).

Uremic rats were observed to have the highest urea concentrations in comparison with the values obtained for the unoperated control and

sham-operated rats. This response could be due to the effect of uremia on the stimulation of nitrogen metabolism or to reduced renal clearance of urea as a result of renal nephrectomy and ligation. The greatly lower body weight gains observed in uremic animals compared to control and sham-operated rats was probably a result of the impaired nitrogen metabolism, as evidenced by the development of azotemia in uremic rats.

The dramatic reduction in both GOT and GPT enzyme activities in uremic rats could be due to direct feedback inhibition of the enzymes themselves, inhibition of synthesis or degradation of the enzymes, or by inhibition of coenzyme activity. Further research will be required in order to determine which, if any, of these mechanisms is responsible for the reduction of both transaminase enzymes to near zero levels in renally insufficient animals. In addition, it is imperative that the agent(s) responsible for causing this effect, likewise, be isolated. Although the present investigation was not designed to answer that question, it seems evident that urea, one of its metabolites, or or possibly some other nitrogenous compound might be likely candidates.

Regardless of the causative agent or the mechanism involved, it is apparent that both GOT and GPT are dramatically reduced in uremic rats. These data suggest that clinical investigations of humans with uremia need to be further researched in order to ascertain whether the present model system in rats might also occur in man, and therefore, be of importance in the clinical diagnosis of renal disorders.

## SUMMARY

Female Sprague-Dawley rats weighing 150-250 g were made uremic by ligation of two-thirds to three-fourths of the left renal artery, followed one week by contralateral nephrectomy. Sham-operated rats had only the left renal artery ligated, as above. All rats of similar weight were pair-fed diets containing 5% protein (casein) or 23% protein (Purina Lab Chow) for three, six and nine weeks.

At each level of protein intake uremic rats gained less weight than their controls, and the sham-operated rats. The urea concentration was higher in both groups of uremic rats, being significantly higher in the group fed 23% protein, while transaminase activities (GOT and GPT) were dramatically lower in comparison with the values obtained for the sham-operated and the unoperated control rats. In addition, the level of both transaminase enzymes were lower in uremic rats fed 23% protein, and the values for the urea plasma concentration were higher than the values obtained for uremic rats fed 5% protein. These observations indicate that time, diet and uremia induction have direct relationships with body weight gains, and urea plasma concentration, but inversely proportional relationships were observed for GOT and GPT activities, with respect to both diet and time. The low activity of the transaminase enzymes found in both groups of uremic rats may be due to an inhibition by urea, or by some other substance(s) present in the uremic serum.

The fact that GPT activity in the sham-operated group fed 23% protein was higher in comparison with the results obtained for the control and uremic rats fed the same diet, may be due to an excessive release of GPT from the damaged ligated-kidney in that group. The results

found in this study also indicate that uremic rats fed 5% protein utilized protein less efficiently than uremic rats fed 23% protein, although in both uremic groups, the use of protein was less efficient than the sham-operated and the unoperated control groups. The finding that uremic rats utilized protein less efficiently than sham-operated and control rats may indicate that marked reductions in protein intakes may be deleterious to the nutritional status of the uremic animals. It should be emphasized, however, that even though low protein diets are not conducive to increases in body weight in uremic animals, the tremendous benefit of reduced serum urea concentrations in those animals may far outweigh the deleterious effects, except in young animals who need higher protein diets for general body growth.

LITERATURE CITED

Aoyama, Y. H. Y. and A. Kiyoshi. 1971 Effect of dietary protein and amino acid in choline deficient diets on lipid accumulation in rat liver. J. Nutr. 101 (6):739-746.

Biro, J. B. and F. Renyi-Vamos. 1965. Non protein nitrogen in normal and uremic tissues. Acta. Med. Scan. 177(2):161-168.

Bloch, K. 1946. The metabolism of (+) arginine in the rat. J. Biol. Chem. 165:469-476.

Borglin, N. E. 1958. Serum transaminase activities and vitamin $B_6$ in pregnancy. J. Clin. Endocrinol. 18:878-881.

Borst, J. G. G. 1948. Protein catabolism in uremia, effect of protein-free diet, infections and blood transfusion. Lancet. 1:824-828.

Bowering, J. D., J. Calloway, S. Margen and A. Kaufman. 1970. Dietary protein level and uric metabolism in normal man. J. Nutr. 100 (2):249-261.

Bull, G. M., A. M. Joekes and K. G. Lowe. 1949. Conservative treatment of anuria in uremia. Lancet. 2:229.

Chan, W., M. Wang, D. Kopple and Marian E. Swendseid. 1974. Citrulline levels and urea cycle enzymes in uremic rats. J. Nutr. 104:678-683.

Changler, C., E. Lieberman and M. A. Holliday. 1974. A rat model for the study of growth failure in uremia. Pediac. Res. 8:109-113.

Chatterjee, G. C., D. Sasmal, N. C. Kar, N. Sasmal, P. K. Roy, and S. K. Banerjee. 1971. The influence of dietary casein on ascorbic acid metabolism in guinea pig. Indian J. Biochem. Biophys. 8(2):94-96.

Chen, L. H. and Abby L. Marlatt. 1975. Effects of dietary vitamin $B_6$ levels and exercise on glutamic-pyruvic transaminase activity in rats tissues. J. Nutr. 105:401-407.

Cohen, G. A., J. A. Goffinet, R. K. Donabedian, and H. O. Coon. 1976. Observations on decreased serum glutamic-oxalacetic transaminase (SGOT) activity in azotemia patients. Ann. Internat. Med. 84:275-280.

Coles, G. A. 1972. Body composition in chronic renal failure. Quart. J. Med. 41:25-47.

Czerniak, Z. 1970. N-substituted amino acids in serum of patients with chronic renal insufficiency. Clin. Chin. Acta. 28(3):403-408.

Daubresse, J. C., G. Lerson, G. Plomteux, G. Rorive, A. S. Luckx, and P. J. Lefebvre. 1976. Lipids and lipoproteins in chronic uremia. A study of the influence of regular haemodialysis. Eur. J. Clin. Invest. 6(2):159-166.

De Olivera, H. L., J. A. Laus-Filho, and S. Sarti. 1966. Prevention of acute immunological lung change by previously induced acute uremia in rats. Int. Arch. Allergy Appl. Immunol. 29(4):384-392.

Edozein, J. C. 1966. The free amino acids of plasma and urine in Kwashiorkor. Clin. Sci. 31:153.

Edmund, A. 1927. Experimental uremia. Arch. Internat. Med. 40(4):548-570.

Giordano, C. 1963. Use of exogenous and endogenous urea for protein systhesis in normal and uremic subjects. J. Lab. Clin. Med. 62(2):231-246.

Giordano, C. R., Esposito, R., de Pascale, C. and De Santo, N. G. 1966. Treatment in renal failure: In Proceding of the III International Congress of Nephrology, Washington, Vol. 3, pp 214, (Ed) E. L. Becker. Basel: Karger.

Glynn, K. P., A. F. Cofaro and C. W. Fowler. 1970. False elevations of serum glutamic transaminase due to para-aminosalicylic acid. Ann. Internat. Med. 72:525-527.

Harrison, T. R. and M. F. Mason. 1937. The pathogenesis of the uremic syndrome. Medicine, 16:1-44.

Herndon, R. F., S. Freeman, and A. S. Cleveland. 1958. Protein requirements in renal insufficiency. J. Lab. Clin. Med. 52:235.

Holliday, M. A. 1972. Caloric deficiency in children with uremia: effect upon growth. Pediatrics. 50(4):590-597.

Kempner, W. 1946. Some effects of the rice diet treatment of kidney disease and hypertensive cardiovascular disease. Bull.New York Acad. Med. 22(7):358-370.

Kolff, W. J. 1951. Forced-high-caloric, low protein diet in the treatment of uremia. Cleveland Clinic Quart. 18(3):145-158.

Kopple, J. D., M. K. Sorensen, J. W. Coburn, S. Gordon, and M. E. Rubini. 1968. Controlled comparison of 20 g and 40 g protein diets in the treatment of chronic uremia. Amer. J. Clin. Nutr. 21(6):553-564.

Leifer, E., L. J. Roth, and L. H. Himpllemann. 1948. Metabolism of $^{14}C$ labelled urea. Science, 108:248.

Lonergan, E. T., M. Sermar, and K. Lange. 1970. Transketolase activity in uremia. Arch. Int. Med. 126:851-854.

Mackay, E. M. and Lois L. Mackay. Enlargement of the adrenal cortex in experimental uremia. J. Exp. Med. 46(3):429-442.

Mitsuko, O., and O. Atusko. 1971. Effects of dietary composition on vitamin $B_6$ deficiency state. I. On the dietary protein levels and the transaminase activities in rats. Vitamins (KYOTO) 43(5):237-240.

Moise, T. S., and A. H. Smith. 1927. The effect of dietary protein on the remaining kidney of adult white rats following a unilateral nephrectomy. Exp. Med. 46(1):27-41.

Muting, D. and D. Basil. 1967. Free amino acids in serum cerebrospinal fluid and urine in renal disease with and without uremia. Proc. Soc. Exp. Biol. Med. 126(3):754-758.

Pasternack, A. 1964. Pulmonary changes in acute experimental uremia in rats. Acta Med. Scan. 176(6):675-679.

Rose, W. C. and E. E. Dekker. 1956. Urea as a source of nitrogen for the biosynthesis of amino acids. J. Biol. Chem. 233:107-121.

Rust, J. H., W. J. Viesk, and L. J. Roth. 1956. Carbon dioxide fixation and urea synthesis in the rats. J. Biol. Chem. 223:671:675.

Sabath, L. D., A. G. Deborah, and F. Marwell. 1968. Serum glutamic oxalacetic transaminase. N. Engl. J. Med. 279:1137-1139.

Schmidt, P.. and I. Andick. 1968. The effect of the carbohydrate component of protein-deficient diet on caloric intake and body weight in the rats. Acta Physiol. Acad. Sci. Hung. 36(3-2):117-124.

Scribner, B. H., B. Fergus, S. T. Boen, and E. D. Thomas. 1965. Some therapeutic approaches to chronic renal insufficiency. Ann. Rev. Med. 16:285-300.

Sharp, J. C. and G. P. Murphy. 1964. Conditioned avoidance behaviour in primates during various experimental uremic states. Nephron, 1(3)172-179.

Stanley, E. G., J. Wacks-Man., Helen I. Glueck, and J. W. John. 1969. Platelet function in renal failure. N. Engl. J. Med. 280(13):677-681.

Synderman, S. E., P. M. Northon, E. Roitman, and L. E. Holt. 1966. The effect of diet on the plasma aminogram. J. Clin. Invest. 45:1075.

Tabor, C. W. 1970. Taber's cyclopedic medical dictionary. 11th Ed. F. A. Davis Co., Philadelphia.

Tulpule, P. G., and S. G. Kshirsagar. 1959. Effect of dietary protein calories and pyridoxine on glutamate-oxalacetate transaminase (GOT) activity of blood. Indian Med. Res. 46(3):452-460.

Wang, M., I. Vyhmeister, J. D. Kopple, and S. E. Marian. 1976. Effect of protein intake on weight gain and plasma amino acids levels in uremic rats. Amer. J. Physiol. 230(5):1455-1459.

Warnock, L. G., J. Stone, and W. Conttad. 1974. Decreased aspartate amino-transferase (SGOT) activity in serum of uremic patients. Clin. Chem. 20(9):1213-1216.

Wright, P., L. Brereton, and D. E. M. Shell. 1970. Effectiveness of modified Giovanetti diet compared with mixed low protein diet. Metab. Clin. Exp. 19(3):201-213.

Wolf, P. P., Dorothy W. Norman C., and A. S. Coulson. 1972. Low aspartate transaminase activity in serum of patients undergoing chronic haemodialysis. Clin. Chem. 16(6):567-568.

Zar, J. H. 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N. J.