COMPARISON OF PROTEIN UTILIZATION IN DIALYZED AND NON-DIALYZED UREMICS

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M.Sc. ABSTRACT Foods & Nutrition

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COMPARISON OF PROTEIN UTILIZATION IN DIALYZED AND NON-DIALYZED UREMICS

Investigations using diets containing controlled protein levels of high biological value ("Giordano-Giovannetti Diet") revealed an improvement in biochemical status and reduction in "uremic" symptomatology in chronic renal failure.

The utilization of urea for protein synthesis while on this Giordano-Giovannetti diet was studied by an N^{15} tracer technique. Incorporation of the N^{15} into plasma proteins was used as the criterion for the use of non-protein nitrogen for anabolic purposes.

Uptake of N^{15} into total plasma protein and albumin-pool was seen in the non-dialyzed uremic subjects (on 0.3 g protein per kg body weight) and the dialyzed subjects (on diets containing 0.7 g protein per kg body weight to compensate for amino acid losses in the dialysate). The greatest degree of incorporation was seen in one of the non-dialyzed uremics. Lesser, but still significant amounts were incorporated by the dialyzed uremics. Only a slight incorporation of the label was found in the plasma protein of the normal subject.

Results suggest that dialyzed as well as non-dialyzed uremics would benefit from a restricted nitrogen diet containing protein of high biological value.

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INTRODUCTION

The nutritional status of patients with chronic renal failure has assumed increasing importance within the past decade. This has been due to several factors. One of the most important was the development of various dialysis procedures which could be used to prolong life in the individual without functioning kidneys. In addition, improvements in tissue matching techniques and in immunosuppressive agents permitted the survival of kidney transplants between unrelated individuals for months or years. Finally, it has become apparent that appropriate dietary manipulation can definitely reduce uremic symptomatology and is a necessary adjunct to the success of a dialysis programme and the preparation of the patient for transplantation.

It had long been recognized that dietary nitrogen was in some way concerned with the well-being and survival of the subject with chronic renal failure, and a succession of dietary regimens based largely on the substitution of fat and/or carbohydrate for protein were used (Schreiner and Maher, 1961).

In 1963, Giordano proposed that quality rather than quantity of protein was important. He and subsequent workers found that individuals with gross impairment of renal function and high levels of nitrogen waste products were able to incorporate urea nitrogen into the tissue proteins when fed minimum amounts of nitrogen in the form of the essential amino acids.

When renal function diminishes to the point where dietary manipulation is no longer adequate, hemodialysis must be considered. This creates additional nutritional problems, such as the intermittent loss through the dialysis membrane of essential nutrients e.g., amino acids (Young and Parsons, 1966) certain water soluble vitamins (Whitehead et al. 1968) and minerals (Triger and Joekes, 1969).

In addition, since levels of non-protein nitrogen are rapidly returned to near normal several times a week, it might be expected that protein metabolism would be altered in the dialyzed patient. Lacking the stimulus of a high urea load these individuals may no longer be capable of utilizing the urea nitrogen for protein synthesis.

The question thus arises: to what extent does the dialyzed patient display the ability to utilize the nitrogen of endogenous urea for protein synthesis and how does this ability compare with that of the non-dialyzed uremic patient?

REVIEW OF LITERATURE

The Normal Kidney

The kidney produces urine and in so doing maintains the composition of the internal milieu in a remarkably steady state. A normally functioning kidney is able to accomplish this even in the presence of a several fold increase or decrease in most of the components of the average North American diet. The ability to maintain an output commensurate with intake involves major adjustments in the excretion of waste products, electrolytes and water.

In addition to its excretory and regulatory functions, the kidney has other roles such as the production of a renal erythropoietin factor (Gordon et al., 1967) necessary for adequate delivery of red blood cells from bone marrow, and the production of renin, which is concerned with the regulation of blood pressure and blood volume.

The anatomic unit in which the diverse events comprising urine formation take place is termed a nephron (Fig. 1). It consists of a glomerulus and tubule. Each normal human kidney contains approximately one million of these functioning units.

Urine formation begins with the production of a protein-free and cell-free ultrafiltrate of blood from the glomerular capillaries (Fig. 2). This glomerular filtrate is further modified in its passage down the tubular system by active, i.e., energy dependent, processes of reabsorption and secretion of both electrolytes

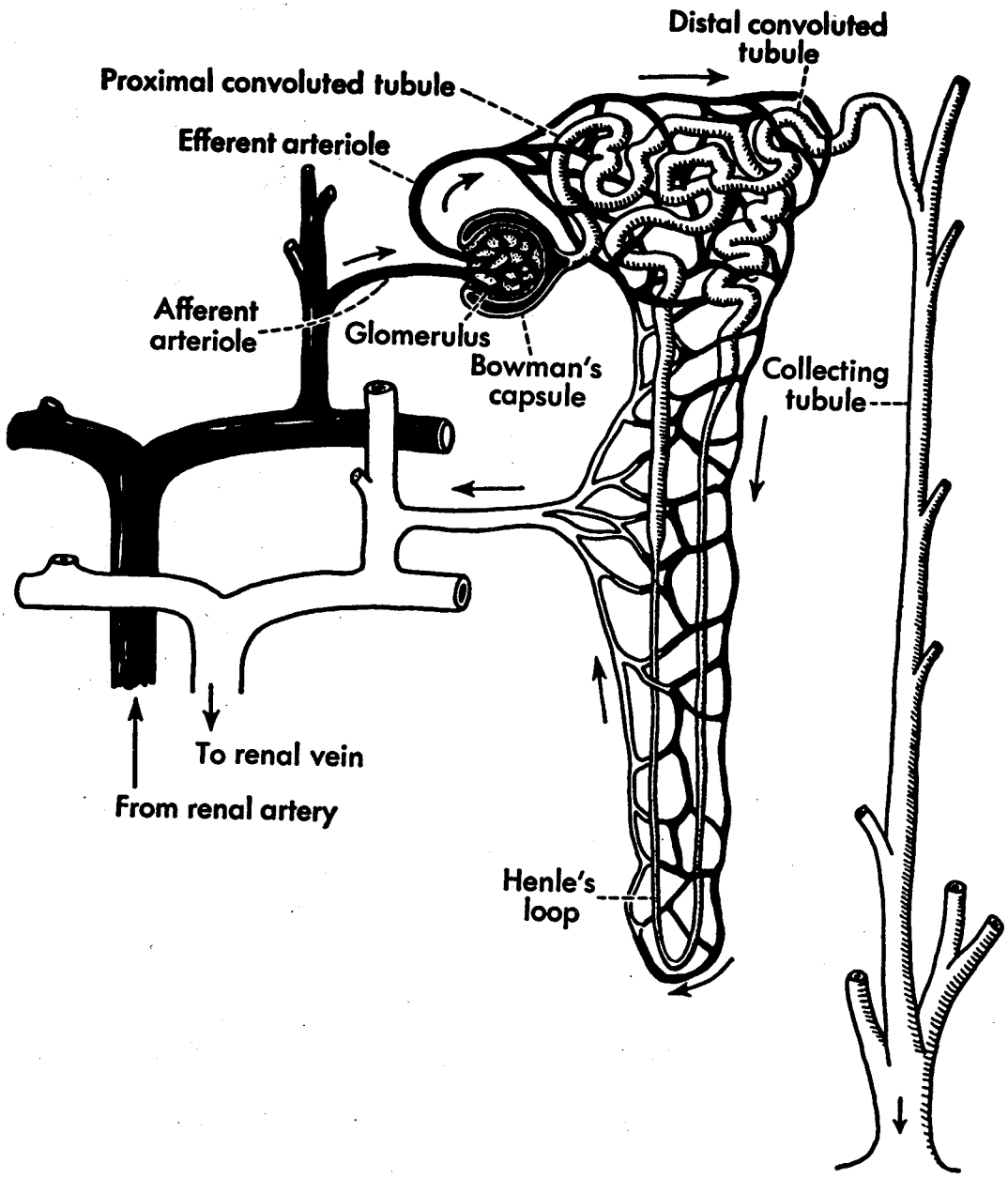

FIGURE 1. A schematic drawing of the nephron showing the glomerulus and tubular system. The blood enters the glomerulus, where filtering occurs, via the afferent arteriole and leaves via the efferent arteriole. (Merck, Sharp and Dohme "Seminar," Vol. 9, No. 3, 1947).

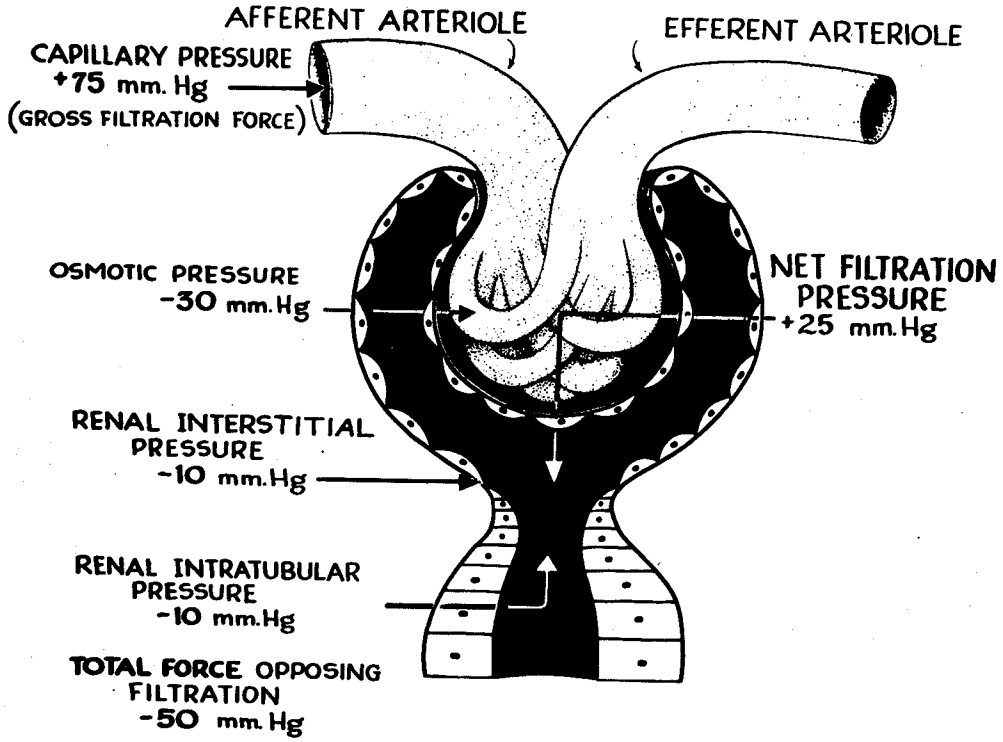

FIGURE 2. Factors affecting the net filtration pressure. (Merk Sharp and Dohme "Seminar," Vol. 9, No. 3, 1947).

and non-electrolytes, and by passive diffusion of water in response to solute gradients developed across the cell membrane at various sites within the tubules.

Of the litre or more of blood delivered to the adult human kidneys each minute, about 120 ml of filtrate is formed at the glomerulus. Most of this filtrate is ultimately reabsorbed leaving a highly modified fluid, the urine, in a volume which may range from 0.5 to more than 30 ml per minute.

The net result of the process of filtration, reabsorption and secretion is to return to the circulation, via the renal vein, blood from which waste products have been largely removed and whose composition is "ideal" for the proper functioning of the body cells at any given moment.

The Diseased Kidney

Chronic renal failure is the functional consequence of progressive disease. The anatomic and functional changes of most disease entities resemble those of the experimental model in which one kidney is removed and at later intervals slices are taken from the remaining organ. In both this experimental model, and in human renal disease, the remaining nephron units appear to function in a qualitatively normal fashion and early show both anatomical and physiological evidence of hypertrophy. Once functioning renal mass is below 30%, further hypertrophy does not occur and loss of tissue is associated with proportional loss of function (Bricker, 1969).

It is at or about this point that the functional impairment becomes sufficient to result in recognizable symptoms and biochemical disturbances.

The excretory ability of the kidney is impaired. In 1947 Addis showed that in normal individuals blood urea levels rose with increasing levels of dietary protein. In renal disease, nitrogen retention reflects decreases in glomerular filtration rate. Substances where excretion is dependent upon filtration without subsequent modification during passage along the tubular system can be expected to show rising plasma concentrations as glomerular filtration rate falls so long as the individual remains in external balance i.e., intake = output. This is true for most end products of protein catabolism such as urea, and may be expressed as follows:

$$Q = K\ GFR \times P$$

where Q represents quantity of urea formed and excreted on a given protein regimen, GFR is the glomerular filtration rate, and P the plasma urea (the concentration in the glomerular filtrate). K is a proportionality constant (Epstein, 1956).

Sodium is freely filtered at the glomerulus. In health about 99% of filtered sodium is subsequently reabsorbed in the tubular system under the influence of the adrenal cortical hormones. Without alteration in filtration the normal tubular system is capable of producing urine in which the sodium excreted can range from zero to several hundred milliequivalents per day in

response to appropriate changes in dietary intake (Black, 1967). The failing kidney is incapable of making these adjustments. Because of some hypertrophy each remaining nephron has a somewhat increased filtration. However, total amount of sodium filtered is reduced and of greater importance, tubular capacity to reabsorb is grossly impaired. The net result is loss of flexibility, and these kidneys are frequently found to excrete relatively fixed amounts of sodium ranging between 20 and 40 milliequivalents per day. An intake of more than 40 milliequivalents may lead to edema and/or hypertension, whereas less than 20 milliequivalents may result in sodium depletion.

Plasma potassium concentration, since this ion speices is actively secreted by tubular cells and is not entirely dependent on glomerular filtration, tends to remain relatively normal as long as urine output exceeds 500 ml per day and acidosis is not severe.

Reduced renal excretion of phosphorus, coupled with enhanced urinary losses of the active metabolites of vitamin D leading to diminished gastrointestinal absorption of calcium and secondary overactivity of the parathyroid glands are common in renal disease of long duration and result in diminished bony mineralization. This may be associated with pain and sometimes with fractures (Slatopolsky, 1969).

Hyperglycemia without glycosuria may occur in renal failure. Briggs et al. (1967) provide evidence suggesting normal production and release of insulin antagonized by a dialyzable nitrogenous

retention substance.

The term "uremia" coined by Piorry and l'Heretier (1840) literally means "urine in the blood" and was used to describe the retention of nitrogenous wastes. It is commonly used to designate the whole symptom complex ensuing with failure of the kidneys to regulate composition and volume of the body fluids (Pitts, 1969).

A variety of disturbances in cellular and subcellular function have been described in the uremic state. In a review of the pathogenesis of uremia by Teschan (1970) the evidence of the sites of dysfunction is discussed. At the subcellular level several enzymatic systems have been shown to be inhibited by the presence of elevated concentrations of a variety of nitrogenous waste products. At the cellular level changes have been shown to occur in erythrocyte homeostasis (Welt et al., 1964). Abnormalities also occur in various organ systems in the uremic. Integrated defects in the whole body are reflected in defective erythropoiesis, carbohydrate, lipid and bone mineral metabolism. These may be caused by biochemical or hormonal derangements detectable at lower levels of biological organization.

Whatever the relative importance of the described disturbances may be, it is now quite clear that many of them can be returned towards normal by appropriate dietary manipulations. By itself diet may have little bearing on the ultimate outcome but may appreciably improve patient well-being and both physical and mental performance over long periods.

The Artificial Kidney

"Artificial kidneys" or more correctly extracorporeal dialysis machines are designed to substitute for some of the functions of the intact kidney. These devices are capable of changing blood composition by ultrafiltration or by simple diffusion but obviously cannot completely supplant the homeostatic role of the normal kidney.

The selective reabsorptive ability of the tubular system in response to varying homeostatic demands of the body is absent, as is the hematopoietic mechanism. Blood pressure control is possible to some extent by the decreased fluid volume and sodium removal. However, the control of blood pressure through the production of renin is absent.

Dialysis, the term used to describe this procedure, basically consists of exposing blood to a solution of ideal composition, physically separated by a semipermeable membrane through which solutes may move in response to appropriate hydrostatic or concentration gradients.

One common type of dialyzer is the Kiil, which consists of three board-like polypropylene structures stacked with two layers of cellophane membranes between each board (Fig. 3). To allow blood to pass between these two membranes, silastic tubes are permanently implanted into a vein and an artery in a limb of the patient (Fig. 4, Fig. 5). At the time of dialysis the "U" tube connecting the vein and artery is removed allowing the blood to

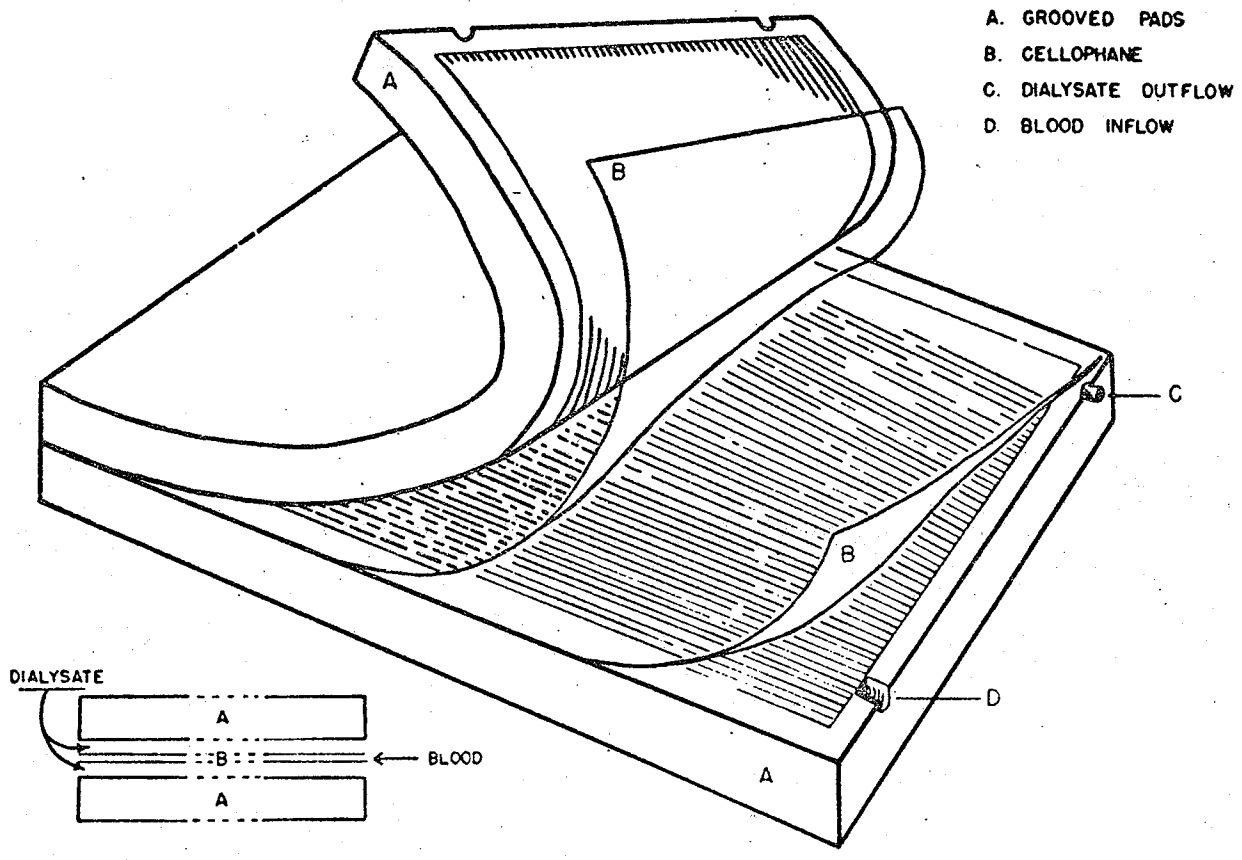

FIGURE 3. Schematic diagram of the dialyzer. Blood is pumped through two cellophane membranes (B) compressed between two corrugated boards (A). The dialysate travels between corrugated surface and cellophane membrane.

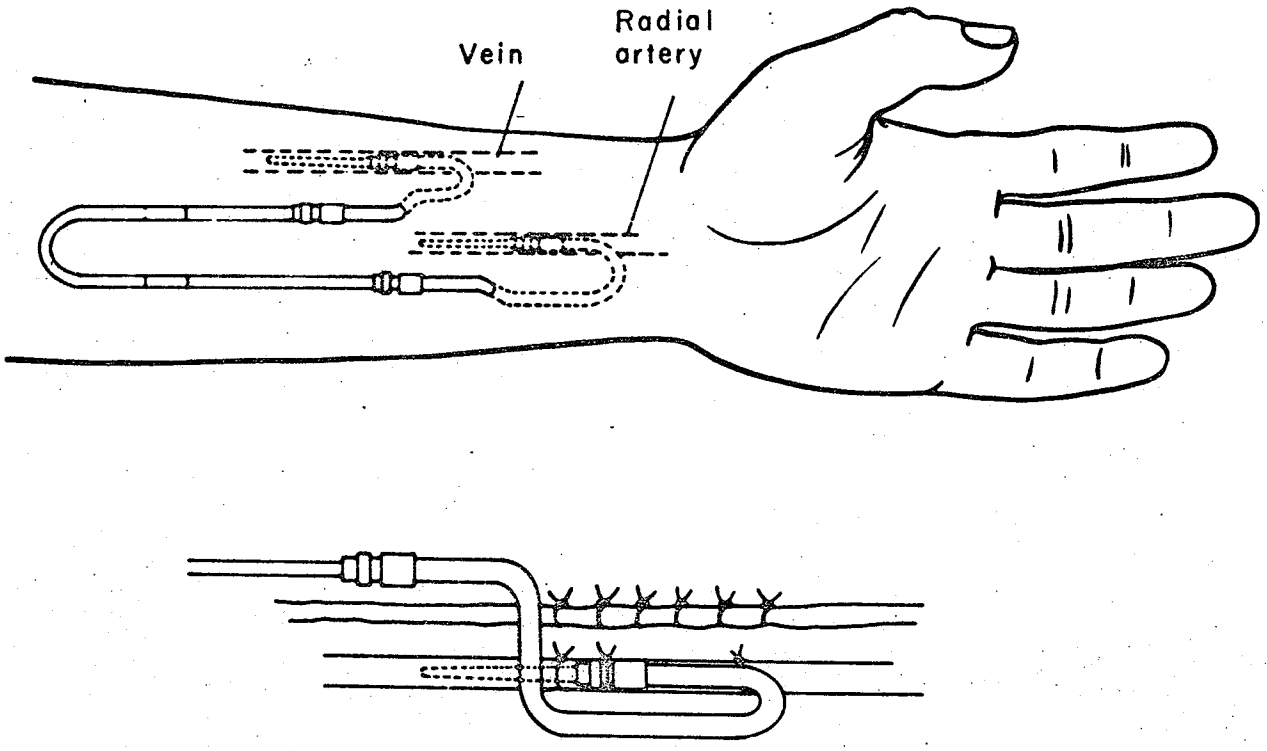

FIGURE 4. Diagram of arteriovenous shunt placed in the arm of the patient (Black, 1967).

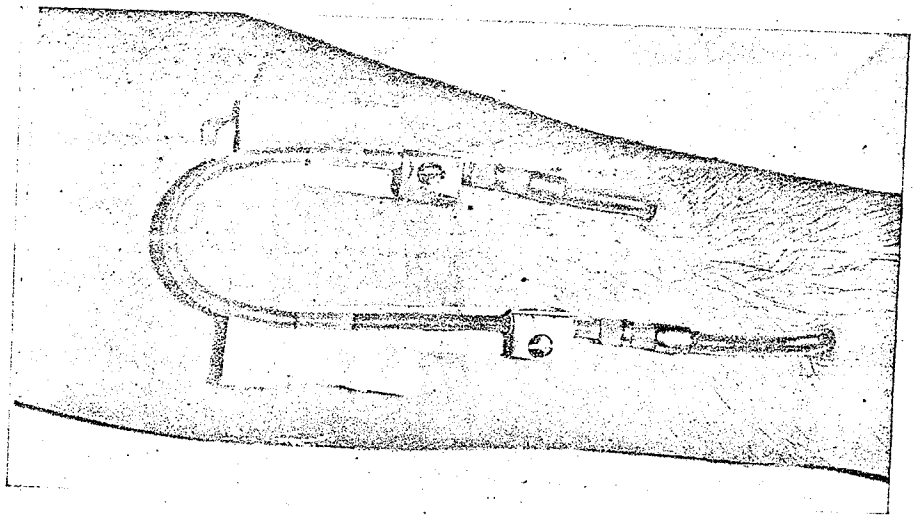

FIGURE 5. Arteriovenous shunt in the arm. The silastic tubes are connected by a "U" tube which is removed at the time of dialysis (Black, 1967).

flow to the dialyzer through a series of tubes connected temporarily for the treatment. The blood passes between the two membranes, returning to the patient through tubes connected to the end of the Kiil.

In the Kiil, the dialysate flows between the corrugated surface of the "boards" and the membrane, thus permitting diffusion of the waste products into the dialysate.

Where no modification of a solute is desired concentration on the dialysate side of the membrane is kept the same as the blood. When removal is desired, the concentration in the dialysate is kept less than plasma and this favourable concentration gradient is maintained by a continuous supply of fresh dialysate.

In the present stage of development, the dialysate fluid is kept relatively simple in composition and is lacking in many of the diffusible blood constituents which may be of considerable nutritional importance. Giordano et al. (1968a) report amino acid losses (including peptides) in the order of 14 to 20 grams in six hours of dialysis.

Due to the loss of these vital amino acids, efforts must be made to insure an adequate supply of protein of high biological value in the diet to replace them as these losses increase the state of protein depletion in the uremic. This in turn may play a direct role in the limited vigor and stamina, as well as bear a relationship to the decreased resistance to infection seen in these individuals.

This presents a challenge to the nutritionist because at the same time the diet must be designed to prevent excessive accumulation of nitrogenous metabolites between dialysis.

Diet Therapy

Diet therapy is the basis of conservative management in chronic renal failure. It is designed to prevent toxic metabolites from rising to levels which interfere with the well-being of the patient.

These dializable metabolites, of which urea, creatinine and uric acid are most often measured, are the by-products of protein metabolism. There are other dialyzable metabolites, some which have not been identified. Hicks et al. (1962) tabulated 94 different specific compounds and indicated evidence for nearly 200. These substances appear to move in parallel with urea, and are possibly more harmful than those which can be measured.

The end products of carbohydrate and fat metabolism (carbon dioxide and water) present few problems in renal disease. The carbon dioxide is simply exhaled via the lungs and the small amount of water is eliminated by lungs, sweat glands and feces.

The history of diet therapy in renal disease has pursued a long course in which there has been no unanimity of opinion. Early in the 19th century a relationship was observed between nitrogen retention products and symptoms associated with kidney disease (Christison, 1829). Attention was thus focussed on the protein content of the diet. Many theories have been advanced for

adjusting both quantity and quality of dietary protein. Emphasis at one time was placed on the type of protein foods recommended. Red meat was thought to contain nephrotoxic extractives while white meat and fish were considered less harmful. Later this was disproven and egg incriminated as an injurious form of protein in patients with renal disease (Fishberg, 1954).

The quantity of protein in the diet has been subject to a wide variety of opinions. Kempner (1945) reported that on a 20-gram protein diet consisting of rice and fruit, azotemic hypertensive patients were able to maintain positive nitrogen balance. Peters and Van Slyke (1946) could see no advantage in restricting protein and recommended 1 gram of protein per kilogram body weight. Borst (1948) then introduced his high fat, high calorie diet. He felt the disadvantages in allowing high levels of protein had been seriously underestimated and recognized the importance of supplying an adequate caloric intake to accompany protein restriction.

Because of the unpalatable nature of this high fat diet with not infrequent accompanying nausea, Bull, Joekes and Lowe (1949) applied the same principle to a high fat, 2500 calorie protein-free diet fed via a stomach tube passed through the nose. All vomitus was collected and refed insuring accurate fluid intake with no loss of fluid or electrolytes. When urine output reached a litre per day, a low protein diet was initiated.

Results of this method of therapy were not successful on a long term basis, largely because of the resulting severe nitrogen

imbalance and endogenous protein catabolism.

In 1953, Kolff suggested that a satisfactory method of disposing of retention products when they cannot be excreted would be through reutilization for protein anabolism. He pointed out that ruminants were known to be capable of utilizing non protein nitrogen and rats were able to use the nitrogen of ammonium salts for growth.

In 1956, Rose and Dekker showed conclusively that urea could be used as a source of nitrogen in the synthesis of non essential amino acids in the rat. Previous workers (Kriss and Marcy, 1940, and Block, 1946) studying urea in rats had concluded that urea was metabolically inert in monogastric animals. Rose and Dekker showed that by limiting the total dietary nitrogen to minimal amounts of essential amino acids, rats would utilize the nitrogen from the labelled urea to form the non essential amino acids, whereas rats fed a normal (18% casein) diet did not utilize the urea nitrogen for synthetic purposes.

Walser and Bodenlos (1959) gave labelled urea intravenously to normal subjects. The labelled ammonia produced from the urease splitting of urea in the gut was found to be reincorporated into newly formed urea.

In 1963, Giordano redirected the approach of dietary treatment in chronic renal failure by showing that azotemic patients were able to utilize urea for protein synthesis if the appropriate conditions were met. The nitrogen was supplied by

minimal amounts of essential amino acids and an adequate caloric content to prevent utilization of protein for energy.

In these studies a nitrogen balance technique was employed. The most important result was the decrease in blood urea nitrogen (BUN), suggesting the utilization of urea for anabolic purposes. As the BUN decreased there was simultaneous clinical improvement.

Richards et al. (1967) studied the utilization of ammonia nitrogen for protein synthesis in normaland uremic subjects. The results of these studies revealed 1) in healthy individuals, the more severe the protein restriction, the greater the incorporation of isotope into the plasma-albumin pool and 2) the uremic subjects (on restricted protein diets) incorporated three and five times as much as the normal individuals on protein restricted diets.

More recently Giordano and his co-workers (1968b) studied the incorporation of N^{15} urea in plasma proteins and their constituent amino acids in normal and uremic subjects on diets containing varying levels of nitrogen. The results of these studies indicated that on the same nitrogen intake, a greater amount of N^{15} was present in the non essential amino acids of the subjects with chronic renal failure than in the normal subjects.

Patients with a higher blood urea showed a greater N^{15} uptake in albumin due to the increased retention of label in these individuals. However, degree of incorporation did not depend solely upon the available label as one subject with an N^{15} blood urea two to three times higher than the normal utilized

urea in the synthesis of the non essential amino acids more than six times as much as the normal. There appears to be a difference between normal and uremic subjects as far as the utilization of urea for protein synthesis is concerned. This could possibly be explained on the basis of the protein depletion of the uremic.

Read et al. (1969) reported results of N^{15} ammonia and N^{15} urea studies which demonstrated the incorporation of N^{15} into blood cells and plasma proteins in malnourished children. This was not observed in the normal controls. Plasma aminograms of uremic subjects reported by Giordano et al. (1968a) were found to be similar to those found in diseases of protein depletion such as kwashiorker as reported by Snyderman et al. (1966). This state of protein depletion might then provide a stimulus for the utilization of nitrogen compounds for protein synthesis.

The mechanism by which this occurs is unknown, but two possibilities have been investigated. The first is ammonium utilization after urea has been decomposed by intestinal bacteria. N^{15} incorporation was studied in a uremic subject given an antibiotic to suppress intestinal flora (Giordano et al., 1968b). The rate of N^{15} utilization was less than subjects given the same diet without the antibiotic. This suggests the importance of bacteria urease in urea nitrogen utilization.

The second possibility is an alternate pathway in urea synthesis as proposed by Cohen and Horowitz (1968). These workers report the increased levels of guanidino succinate, a substance

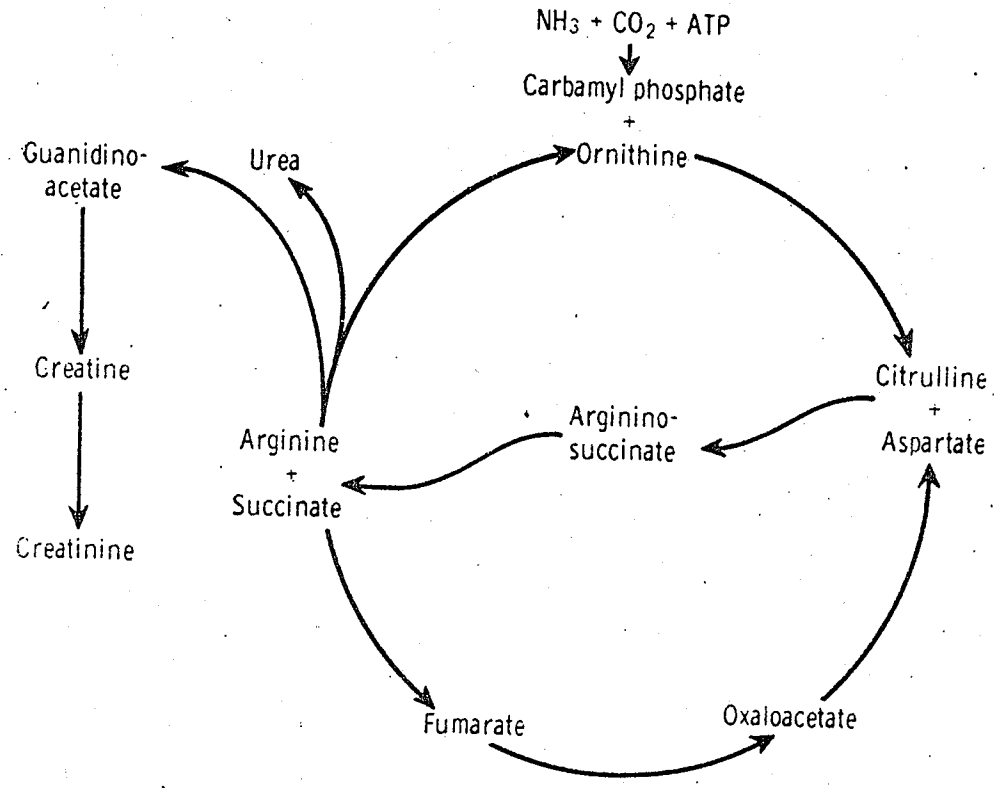

FIGURE 6. The urea cycle.

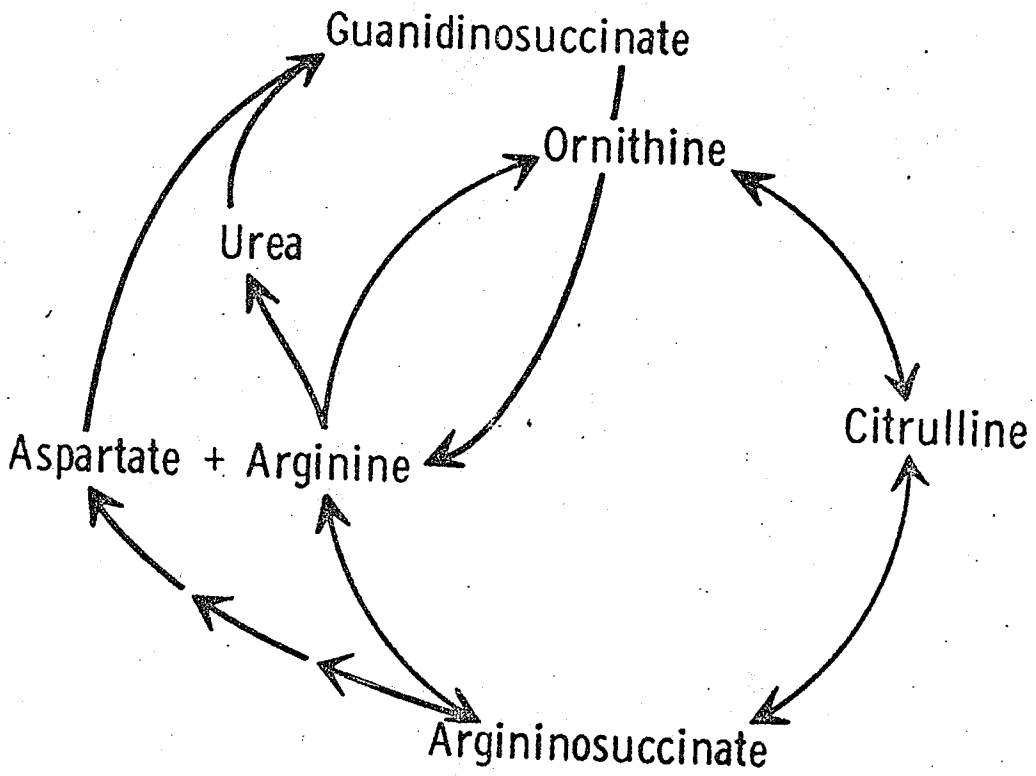

FIGURE 7. Altered urea cycle - reutilization of urea as proposed by Cohen and Horowitz (1968).

structurally analogous to urea, in azotemic patients. Studies performed revealed that the elevation of plasma urea might be the stimulus for production of guanidino succinate in the liver, either as an alternate pathway for urea biosynthesis or an intermediate in the reutilization of urea for synthesis of non essential amino acids. Reutilization of urea implies a reversal of the urea cycle which is thermodynamically possible.

The urea cycle may be seen in Fig. 6 while an alternate pathway suggested by Cohen is illustrated in Fig. 7. A subcycle is set up, the net effect being the reversible breakdown of arginine which allows reutilization of urea in anabolic pathways. Giovannetti and Maggiore (1964) tested these hypotheses by substituting food sources of protein for the synthetic essential amino acids. They compared the effects of a basal protein-free diet supplemented in one of two ways: 1) essential amino acids in recommended daily amounts according to Rose (1957) or 2) naturally occurring protein in the form of whole egg or egg albumin.

Their results showed that on the basal protein-deficient diet, blood urea values fell to levels inversely proportional to residual renal function. When the essential amino acid mixture or egg protein was added to this protein-deficient diet there was no significant increase in blood urea levels observed. These investigators also noted the decrease in clinical "uremic" symptoms associated with the reduction of blood urea levels.

As a result of these investigations, the dietary principles

embodying specific levels of high quality protein have been applied fairly extensively with the foods adapted to the palate of other nationalities.

In Britian, Berlyne and Shaw (1955) studied the use of this diet in terminally ill uremic patients. They compared the clinical symptoms associated with this diet and those observed in patients on the standard low protein (40 grams) diet conventionally used.

The striking difference noted in the patients on the Giordano-Giovannetti type diet was the disappearance of the gastro-intestinal symptoms so commonly associated with uremia. This could be due to associated decrease in endogenous urea circulation with fall in interstitial levels of urea and ammonia.

In North America, Franklin and his co-workers (1957) adapted a "selected protein" diet to appeal to the American palate. They studied 34 patients with chronic renal failure of variable etiological origins. Many of these patients had been following a diet containing 40 grams of protein per day prior to the selected protein diet.

The diet consisted of 13 to 14 grams of animal protein to supply the appropriate quantities of the essential amino acids. Five to six grams of vegetable protein were added to supplement the diet. Calories were supplied by sugar, fat and wheat starch products. The sodium in the diet itself was approximately 40 mEq per day. With the addition of salt it was increased to 100 mEq per day. The potassium content varied between 40 and 50 mEq per day.

Of these 34 patients, seven (22%) refused to adhere to the diet because of its unpalatable nature. Of the 27 who were able to adhere to it, amelioration of the common symptoms associated with terminal renal failure, such as anorexia, nausea, weakness, fatigue and lethargy, was observed in 23. Eleven were able to return to work and three were able to resume household responsibilities.

Decreases in blood urea nitrogen levels by an average of 50% occurred in all patients. Serum creatinine levels remained unchanged.

Their clinical evaluation was based on the amount of relief from previous uremic symptoms and total duration of remission. Using these criteria, 13 were ranked as poor, four fair and ten good to excellent. The patients ranking in the "poor" category tended to have lower glomerular filtration rates (less than 1 ml/min) or could not adequately follow the diet. The duration of response to the diet depended largely upon factors which determine the progress of the disease.

Snyder and Merrill (1966) reported the use of a similar diet containing 22 grams of protein chiefly of egg and vegetable origin. Thirty calories per kg of body weight were provided by fat and carbohydrate. Within a week of initiation of this diet, patients experienced relief of symptoms.

The major drawback to this diet is the dearth of commercially available products which may be used. The implementation of this

selected protein diet is a challenge to the skill of any culinary artist.

When renal function diminshes to the point where dietary treatment alone is no longer sufficient, other supportive methods of treatment must be sought. In recent years, chronic intermittent hemodialysis has been made available to an increasing number of terminal uremics.

To date there is no known "best diet" for treatment of the patient on dialysis. Pendras (1968) feels that patients fare better on diets containing a relatively high (80 grams) protein intake than those taking diets containg 60 grams of protein.

To allow for this increased protein intake, dialysis time was increased from two to three times per week. The additional dialysis time seemed to be a small price to pay for the improvement in general well-being and increased ease in handling the diet while travelling, eating out, etc. as far as the patients were concerned.

Ginn and his co-workers (1968) studied nitrogen balance in subjects being maintained on intermittent hemodialysis. These studies were designed to define the quantity and quality of protein required 1) to obtain nitrogen balance, 2) minimize azotemia, 3) reduce the time of dialysis. Studies were performed using varying levels of protein of high and low biological value. The results indicated that patients receiving 0.75 grams of good quality protein (i.e., egg and meat sources) per kg body weight with adequate calories were able to maintain neutral or slightly

positive nitrogen balance with normal serum albumin concentrations and minimum increases in blood urea nitrogen between dialysis. When egg and milk supply the protein, 0.63 gm/kg is sufficient to achieve the same results (Kopple et al., 1969).

If azotemia and the associated uremic symptomology could be minimized, then total dialysis time could be reduced and the resulting nutritional losses decreased. By reducing dialysis time per patient, more patients could be treated because the availability of time, equipment, and technical personnel are the chief limiting factors of use of the artificial kidney.

OBJECTIVES OF STUDY

The present study was designed to 1) confirm the reports in the literature by the use of N^{15} urea that endogenous urea can be used for protein synthesis in the non dialyzed uremic and 2) investigate the extent to which the dialyzed patient displays this ability as his chemistry returns to normal in the face of the intermittent "normalizing" effect of dialysis.

EXPERIMENTAL

Preliminary Study

In a preliminary study ten uremic outpatients were placed on modified protein diets following the principles of Giordano-Giovannetti. These patients were instructed to eat high quality protein in the form of milk and egg. An additional six grams of protein was supplied by vegetables to improve the acceptability of the diet (see Table I). Additional calories were supplied by the fat and carbohydrate foods.

Blood urea nitrogen and creatinine levels were measured by methods adapted for determination by the Auto Analyser[1].

Plasma Protein N^{15} Incorporation

Six subjects were then selected for the subsequent study, including one normal volunteer. Of the five subjects with chronic renal failure, three were being treated by intermittent hemodialysis and the remaining two controlled by diet alone. A summary of clinical data may be seen in Table II.

Hemodialysis was accomplished using a Kiil dialyzer with a single pass system of warm dialysis fluid. Periods of dialysis varied as seen in Table II.

The subjects were studied as outpatients carrying on normal duties during this period. All of the food to be eaten was prepared and served by the investigator.

A constant diet using a four-day cycle menu pattern was

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1. Technicon Instrument Corp., Ardsley, N.Y.

TABLE I. MODIFIED GIORDANO-GIOVANNETTI DIET

Foods To Be Included Daily

Milk	240 g
Egg	One
Whipping cream	120 g
Vegetables	To equal 6 g protein

Foods Which May Be Eaten As Desired

Wheat starch[1] products - Bread
Pastries

Cornstarch

Tapioca

Low protein pastas[2]

Low protein fruits

Jam, jelly

Sugar, syrup

Hard candy, gum

Beverages - Carbonated
alcoholic

Herbs and spices

Butter, vegetable oils

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1. MacDowell Bros., Brockville, Ont.

2. A Proten, Carlo Erba (U.K.) Ltd., London, England.

TABLE II. SUMMARY OF CLINICAL DATA

Subject	Clinical Diagnosis	YoB	Sex	C_{Cr} ml/min	Dialysis Time
I.G.	Normal	1949	M	149	---
K.H.	Chron. G. Neph.	1935	M	13	---
N.S.	Chron. G. Neph.	1951	F	5	---
Y.Y.	Chron. G. Neph.	1950	M	2	2/week - 8 hr.
G.N.	Chron. Pyeloneph.	1891	M	8	1/week - 10 hr.
C.R.	Chron. Pyeloneph.	1941	M	*	3/week - 7 hr.

* Hydroureters: clearance measurements not valid.

planned for each individual. A detailed diet history was taken prior to the initiation of the study and food preferences ascertained to ensure acceptability of the diet.

The study was conducted for a 21-day period, including a seven day equilibrium period. In the case of the normal volunteer, due to his normal high dietary protein intake, it was felt that a longer period of adaptation would be required to reach nitrogen equilibrium. A 14-day equilibrium period was thus employed extending the total experimental period in this subject to 28 days.

The Diet

The diet for the non-dialysed subjects, as designed for the subjects in the preliminary study, contained 0.32 g egg and milk protein per kg body weight. Five grams of vegetable protein was added to enhance the palatability of the diet. Dialyzed subjects received 0.63 g egg and milk protein per kg body weight plus the five grams of vegetable protein. A sample of the four day cycle of patient C. R. may be seen in the appendix. In addition to the diet, the patients received a multivitamin tablet.

An effort was also made to arrange the diet to fit acceptable meal patterns as far as the individual was concerned. Breakfast usually followed a fairly standard pattern of fruit, cereal, toast and coffee. Duplication of this presented few problems as bread[1] made with wheat starch flour could be

1. Adapted from Bailey and Sullivan (1967).

substituted for the regular wheat flour bread. Low protein cereal, e.g., puffed rice was used if desired for cereal.

For lunch, an egg (cooked in a variety of ways) served with toast or as a sandwich with wheat starch bread was acceptable when served with fruit or special dessert.

The evening meal, however, presented a greater challenge. Most North Americans are accustomed to meat meals with unlimited amounts of vegetables. In designing this meal an effort was made to take advantage of low protein pastas and the lower protein-containing vegetables.

Wheat-starch products were used to supplement this diet. Muffins, cookies and fruit breads were added, both for "fill value" and caloric value. The remaining calories were supplied by sugar, jams and jellies, soft drinks and fats and oils.

Sodium and potassium levels were based on requirements of the individual as determined by 24-hour urine analysis prior to initiation of the study. These may be seen in Table III.

Following the equilibrium period, each subject was given 14 mg N^{15} per kg body weight in the form of N^{15} urea[1]. It was dissolved in water and divided into three or four portions and administered orally on day one.

Blood samples for measurement of N^{15} incorporation were drawn in tubes containing potassium oxalate and frozen until analyzed. The plasma was dialyzed against running tap water for

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1. Office National Industriel de l'Azote, Toulouse, France.

TABLE III. NUTRIENT INTAKE AND WEIGHT CHANGE.

Subject	Weight kg		Protein grams		Protein total g/kg	Sodium mEq	Potassium mEq	Calories /kg
	Before	After	Animal	Vegetable				
I.G.	82.0	80.0	26.0	5.0	.38	140	54	30
K.H.	64.1	64.4	19.3	5.0	.38	40	30	47
N.S.	48.5	48.5	14.8	5.0	.41	80	48	50
Y.Y.	63.5	63.2	40.2	5.0	.71	44	50	37
G.N.	63.1	63.1	39.1	5.0	.70	37	46	27
C.R.	61.4	62.2	38.0	5.5	.71	87	66	54

13 hours to remove non-protein nitrogen and other low molecular weight substances.

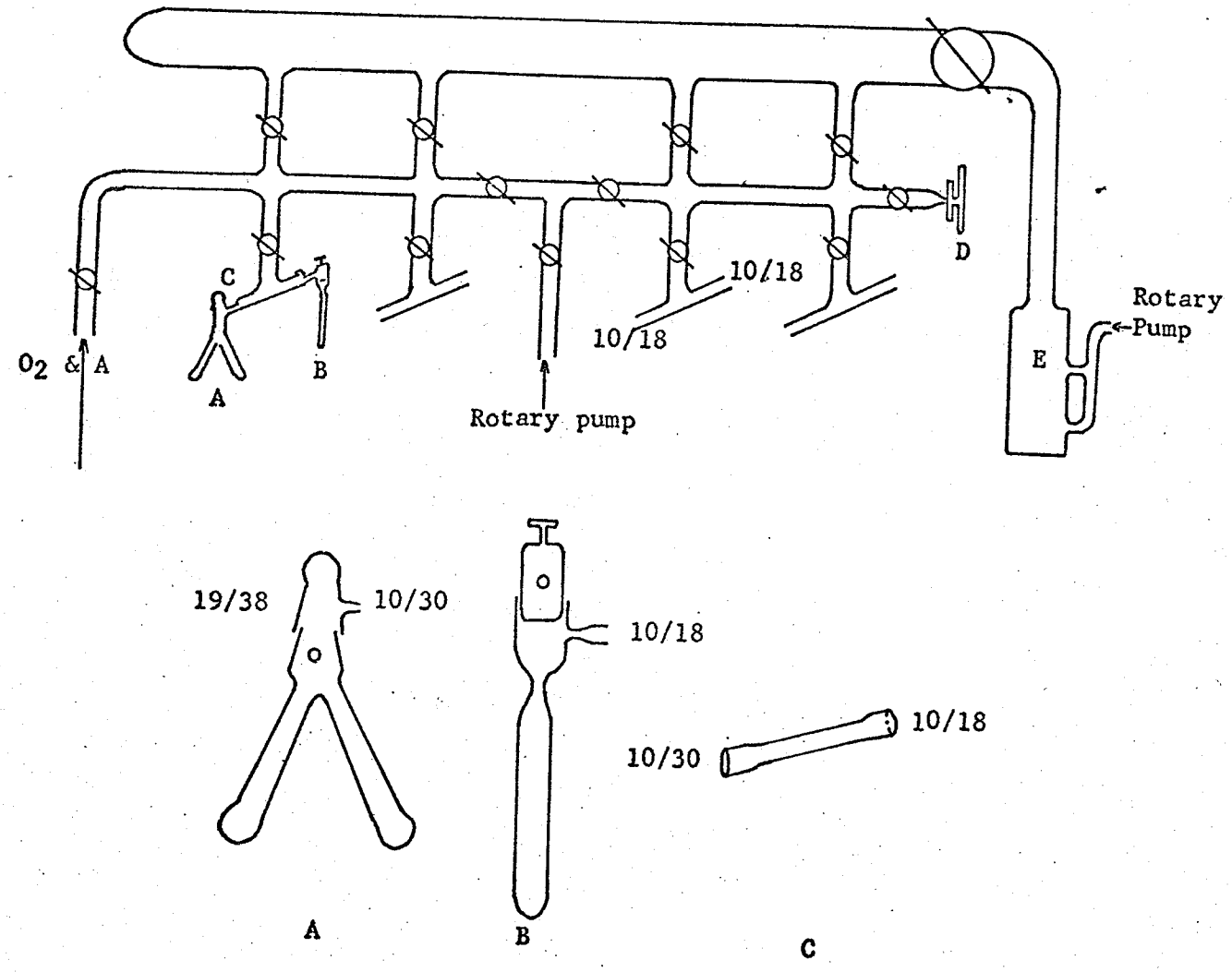
Samples to be analyzed for albumin N^{15} incorporation were prepared by the method described by Debro et al. (1957). To 1.0 ml of plasma, 19.5 ml trichloracetic acid was added, the solution mixed and centrifuged for 30 minutes at 15,000 rpm. The supernatant containing the albumin was aspirated, then evaporated down to approximately 1 ml.

Plasma and albumin samples were then subjected to the standard micro Kjeldahl procedure for digestion and distillation (A.O.A.C., 1965). This method was adapted for our use by trapping the ammonium in 0.05 N HCl. The distillate was evaporated to approximately 10 ml. All Kjeldahl procedures were done in duplicate.

Preparation of Nitrogen Gas

The conversion of ammonium to nitrogen gas was accomplished by treating the sample with alkaline sodium hypobromite in a vacuum system (Fig. 8). The gas receiving bulb (B) was prepared by sealing securely with high vacuum grease and attached on the 10/18 adaptor (Fig. 8).

To one arm of the Rittenburg vessel (A), 3 ml of the sample was added and to the other arm 2 ml of hypobromite solution. Care was taken to prevent mixing the two solutions at this point. The Rittenburg vessel was then affixed to the apparatus on the 10/30 adaptor and immersed in liquid nitrogen. The system was



A = Rittenberg reaction vessel
B = Gas sample container
C = 10/30-10/18 adaptor
D = Virtis micro-McLeod gauge
E = Two stage mercury diffusion pump
⊗ = High vacuum stopcocks

FIGURE 8. Schematic diagram of the vacuum line and reaction vessels.

evacuated completely as indicated by the mercury pressure shown on the McLeod guage (D).

The Rittenburg vessels were then closed and the contents thawed. At this point the solutions were mixed by rotating the vessel, allowing the nitrogen containing solution to flow into the hypobromite solution. The Rittenberg vessel was again immersed in the liquid nitrogen to freeze out water vapour and gaseous byproducts of hypobromite oxidation. The nitrogen gas was then collected in the gas sample bulbs, and the N^{28} N^{29} abundance was measured in the mass spectrometer, MAT Model GD-150. A sample of the graph obtained may be seen in Appendix B.

Calculation of N^{28} N^{29} Abundance

Isotopic abundances are expressed in terms of atom per cent of the isotope. The measured ratio (R) is that of the ion currents corresponding to mass 28($N^{14}N^{14}$) and mass 29($N^{14}N^{15}$) (Bremner, 1965).

$$R = \frac{(N^{14}N^{14})}{(N^{14}N^{15})}$$

The definition of atom per cent N^{15} (A) is:

$$A = \frac{\text{Number } N^{15} \text{ Atoms} \times 100}{\text{Number } N^{14} \text{ Atoms} + \text{Number } N^{15} \text{ Atoms}}$$

$$\text{or} \qquad A = \frac{(N^{14}N^{15}) + 2(N^{15}N^{15})}{2(N^{14}N^{14}) + 2(N^{14}N^{15}) + 2(N^{15}N^{15})} \times 100$$

It is not necessary to measure the ion current corresponding to mass 30 ($N^{15}N^{15}$) to determine N^{15}. The term $N^{15}N^{15}$ can be eliminated because N_2 is in equilibrium with regard to the

reaction

$$B = \frac{(N^{14}N^{15})^2}{(N^{14}N^{14})(N^{15}N^{15})} = 4$$

By combining the equations A and B

$$\text{Atom per cent } N^{15} = \frac{100}{2R + 1}$$

RESULTS

1. Preliminary Study

Table IV shows the effect of modification of the quality of dietary protein as proposed by Giordano-Giovannetti on the clinical and biochemical features of chronic renal failure. Nine of the subjects had been experiencing "uremic" symptomatology such as nausea, frequent vomiting and disturbed taste sensations. On institution of this diet these individuals reported disappearance or reduction of all these symptoms, plus an increase in well-being and improvement in mental alertness. Subject KH was asymptomatic throughout.

Weight was reasonably well maintained in most individuals. A drop in BUN was observed in the face of continuing reduction in function as evidenced by the rise in plasma creatinine levels. Lonergan and Lange (1968) reported an average decrease in BUN levels of 57% on a modified Giordano-Giovannetti diet. The mean value of decreases in this study was 41%.

Figure 9 represents the course of disease in patient KH. The onset occurred in 1955 (Period 1) as a result of acute glomerulonephritis which progressed to chronic renal disease, confirmed by biopsy. In December of 1967, a period of uncontrolled blood pressure caused a rather sudden decline in renal function. At this time (Period 2) he was placed on a diet containing 50 grams of protein. His BUN at this time was 50 mg per 100 ml with a creatinine clearance of 35 ml per minute.

TABLE IV. EFFECT OF MODIFICATION IN QUALITY OF DIETARY PROTEIN IN SUBJECTS WITH CHRONIC RENAL FAILURE

Subject	Age	Diagnosis	Duration of Modified Diet Months	Body Wt kg		BUN mg/100 ml		P. Creatinine mg/100 ml		Relief of Symptoms
				B	D	B	D	B	D	
L.E.	49	pn	2	83.5	83.5	122	78	13	15	++
J.L.	40	pn	2	64.0	61.0	120	82	7	8	++
J.L.	38	pn	2	42.5	42.5	130	96	9	7	++
M.H.	47	gn	4	48.0	45.5	170	120	19	20	+
P.P.	34	gn	3	59.0	60.0	100	38	15	15	++
R.D.	52	pn	2	57.0	56.5	104	53	7	9	++
G.S.	59	pn	10	70.0	70.5	120	50	7	13	+
F.B.	59	pn	12	51.5	50.0	80	45	6	6	++
W.A.	35	gn	4	60.0	59.0	125	69	7	7	+
K.H.	36	gn	12	63.3	64.5	45	20	5	6	o
				Means		112	65	9.5	10.6	

Total protein, 25-30 g/day and caloric intake, 2000 C. remained constant prior to (B) and during (D) ingestion of a diet in which the bulk of protein intake was derived from essential amino acid sources. pn = pyelonephritis; gn = glomerulonephritis.

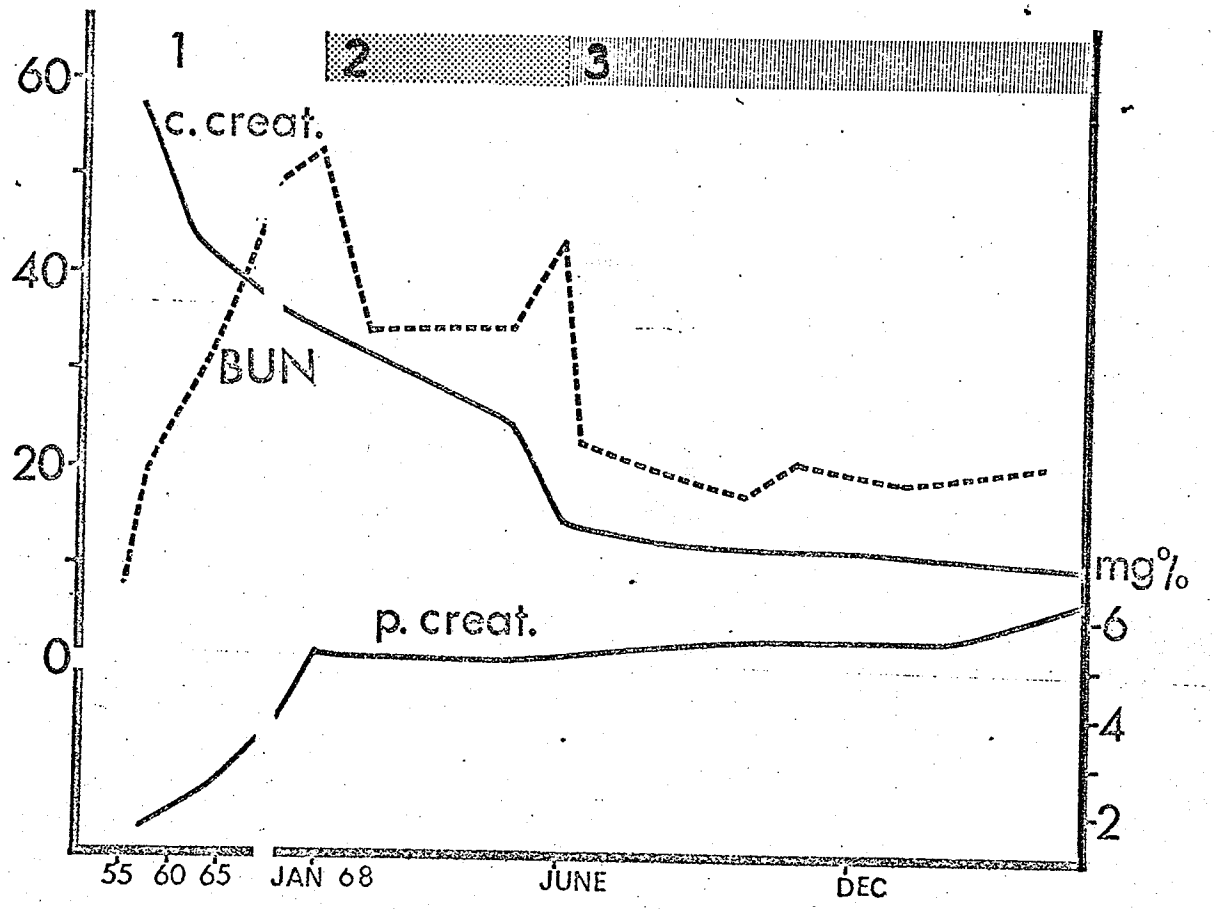


FIGURE 9. Progression of disease and effect of dietary protein modification in Subject KH
c. creat. = creatinine clearance (expressed in ml/min)
BUN = blood urea nitrogen (expressed in mg/100 ml)
p. creat. = plasma creatinine (expressed in mg/100 ml)

A further decline in renal function occurred due to a superimposed infection in April of 1968. At this time he was placed on a Giordano-Giovannetti diet which resulted in a fall in BUN to within the limits of normal (Period 3), and creatinine clearance appeared to stabilize between 10 and 13 ml per minute. His plasma creatinine showed a progressive tendency to rise.

The results of this study showed that a reduction in BUN, which was associated with marked clinical improvement, could be achieved by modifying the quality as well as the quantity of dietary protein as recommended by Giordano (1963) and Giovannetti (1964).

2. Utilization of N^{15} Urea

The natural abundance of N^{15} in the plasma of the six subjects yielded a mean value of 0.368% $\pm$ 0.002 as shown in Table V. This was substracted from values obtained and expressed as N^{15} atom % excess.

Data for N^{15} labelling of the plasma protein and albumin fractions at time intervals following the administration of oral N^{15} urea expressed as percent excess over naturally occurring levels are shown in TableVI. Corresponding time plots for each individual are shown in Figs. 10 to 15. The composite data are plotted in Fig. 16 and 17.

The peak time of label incorporation into plasma proteins was seen to occur earliest in the subject with normal renal function. This peak, however, was at an appreciably lower level than that observed in the subjects with renal failure.

TABLE V. MEAN VALUE DETERMINATION OF NATURAL ABUNDANCE OF N^{15} IN DIALYZED BLOOD PLASMA OF SUBJECTS

Subject	Sample	Atom % N^{15}
IG	A	0.365
	B	0.367
NS	A	0.367
	B	0.369
KH	A	0.366
	B	0.367
CR	A	0.366
	B	0.366
YY	A	0.368
	B	0.373
GN	A	0.370
	B	-----*

Mean Atom % N^{15} = 0.368.

Standard error of mean = 0.002.

* Insufficient sample.

TABLE VI. THE N^{15} ATOM PERCENT EXCESS IN TOTAL PLASMA PROTEIN AND ALBUMIN FRACTION

Subject	Time* (Hours)								
	48	60	80	120	150	180	240	300	336
				PLASMA					
I.G.	.007	.018	.013	---	.008	---	.004	---	.007
N.S.	---	.022	.020	.025	---	---	.032	.031	.024
K.H.	.047	---	.070	---	.099	.066	.031	.028	---
C.R.	.034	.037	.046	.049	---	---	.039	.043	.027
G.N.	.036	---	.043	---	.044	.047	.038	.036	.037
Y.Y.	---	.050	.054	.053	---	---	.048	---	.044
				ALBUMIN					
I.G.	---	.008	---	---	.006	---	.011	---	.008
N.S.	---	.014	.012	.019	---	---	.027	---	.017
K.H.	.016	---	.086	---	.050	.028	---	.015	---
C.R.	---	.046	.035	---	---	---	.032	---	.032
G.N.	.032	---	---	---	---	---	.031	.032	.034
Y.Y.	---	.051	---	---	---	---	.040	---	.042

* Times (hours) were rounded to nearest convenient whole number.

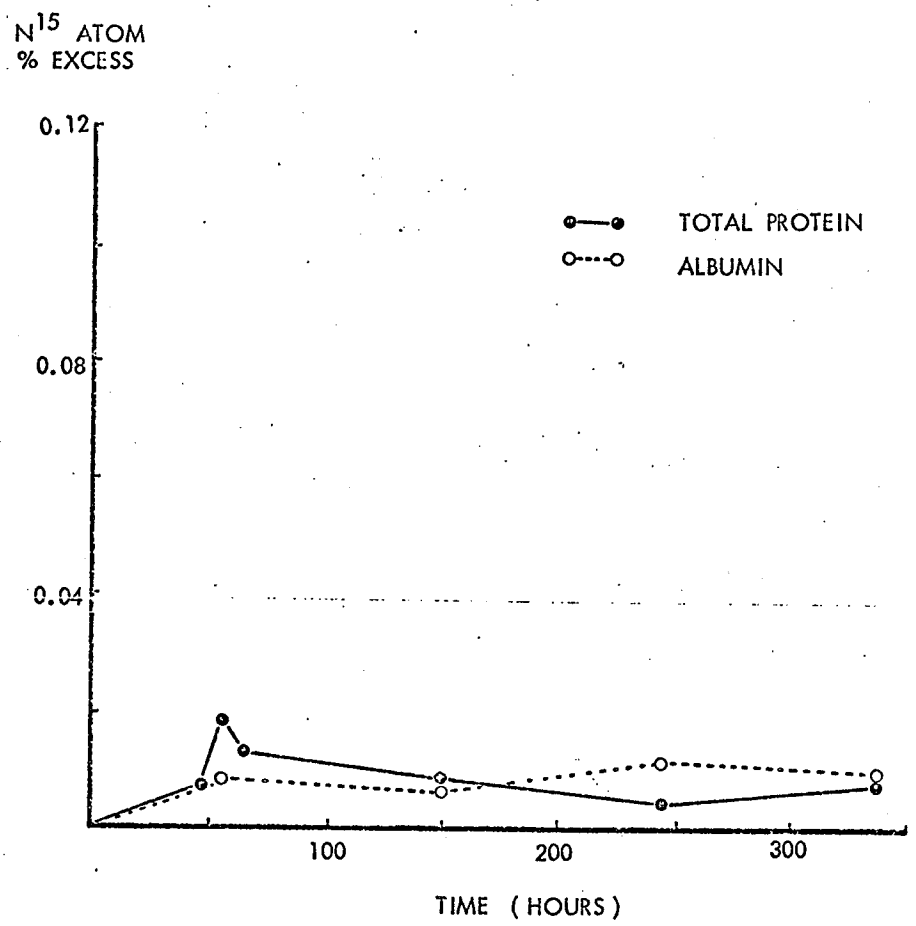


FIGURE 10. N^{15} incorporation into total plasma protein and albumin in a normal subject (IG) on a diet containing minimal amounts of high quality protein.

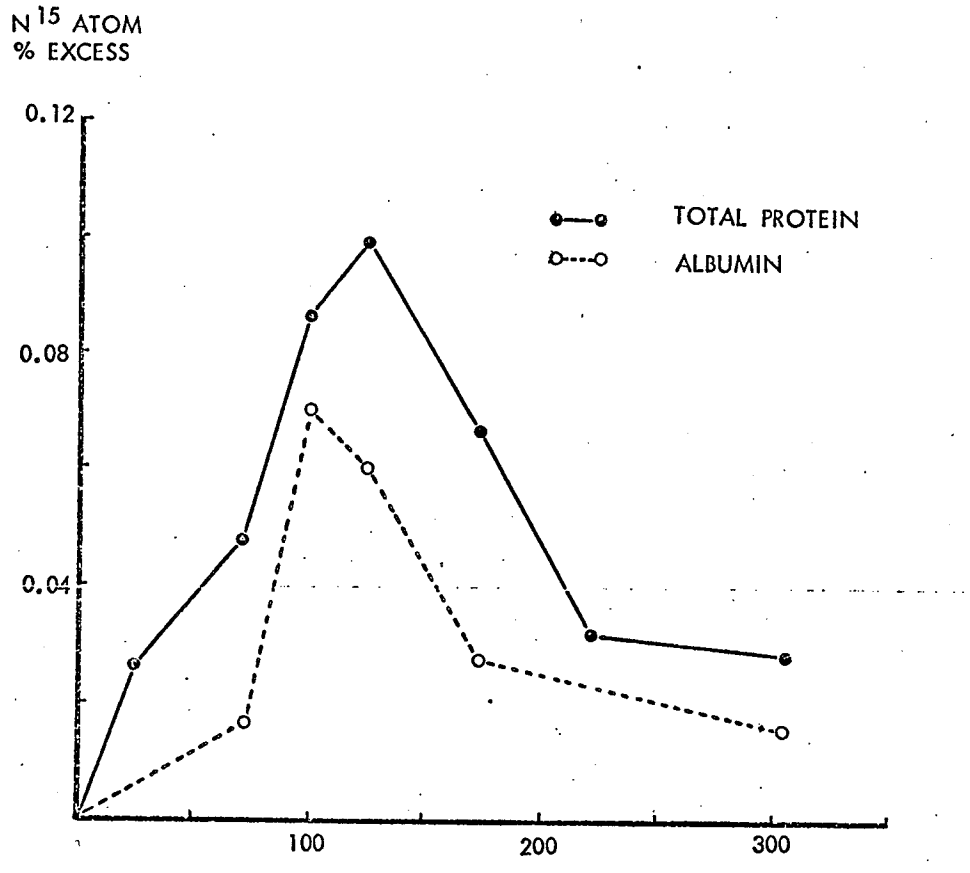


FIGURE 11. N^{15} incorporation into total plasma protein and albumin in a non-dialysed chronic uremic subject (KH) on a diet containing minimal amounts of high quality protein.

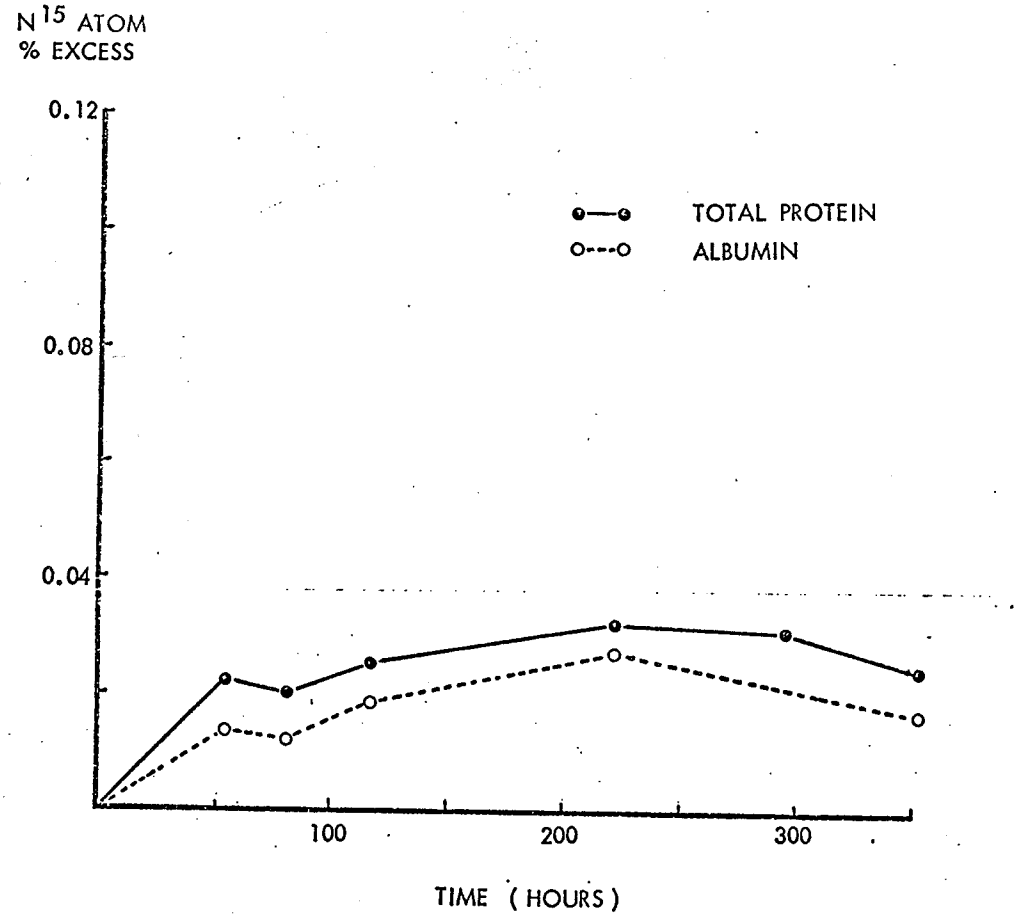


FIGURE 12. N^{15} incorporation into total plasma protein and albumin in a non-dialysed chronic uremic subject (NS) on a diet containing minimal amounts of high quality protein.

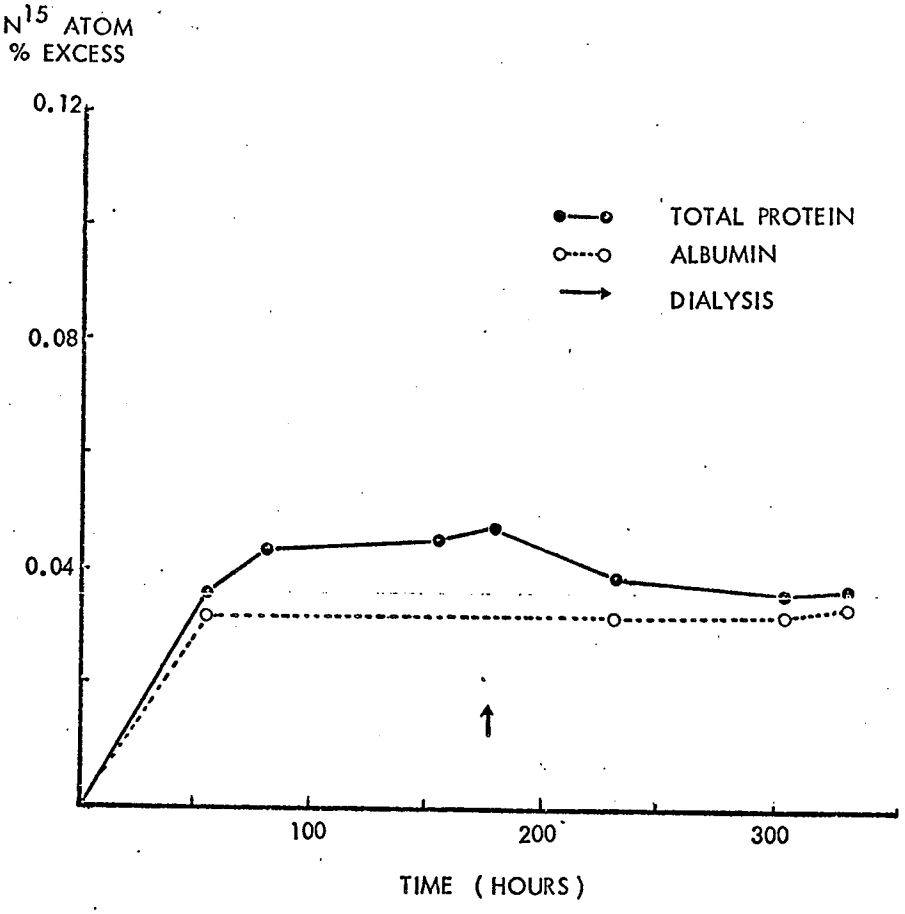


FIGURE 13. N^{15} incorporation into total plasma protein and albumin in a chronic uremic subject (GN) on intermittent hemodialysis on a diet containing minimal amounts of high quality protein.

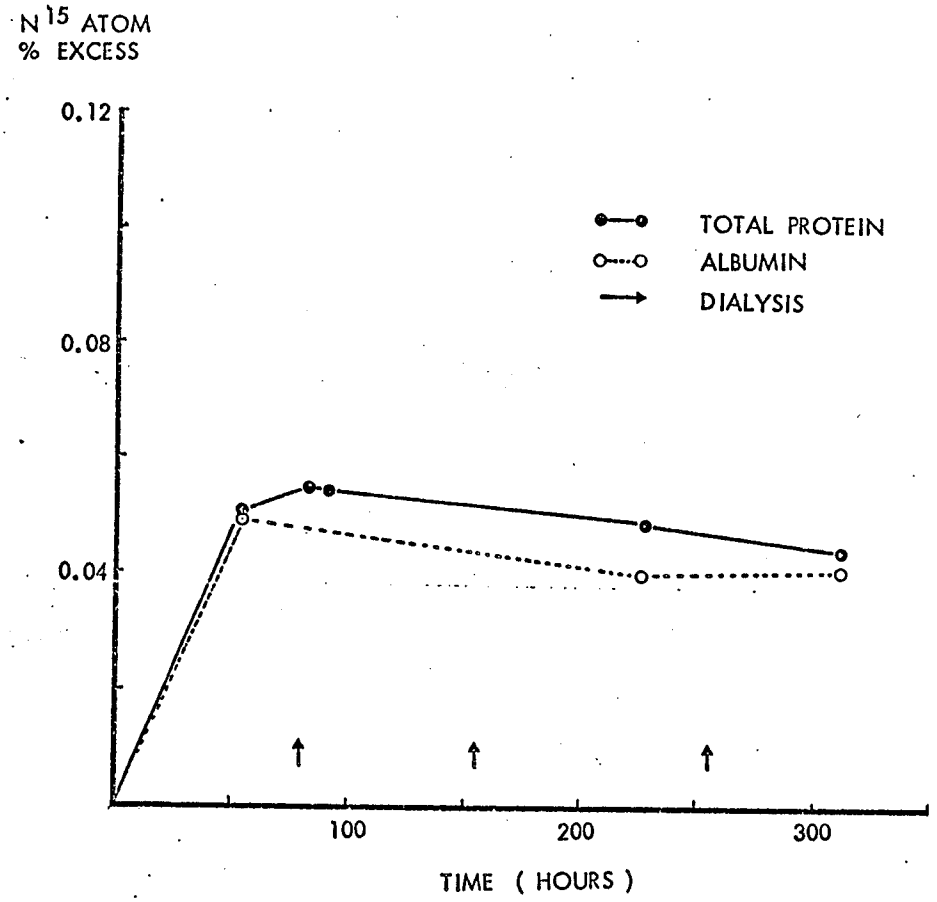


FIGURE 14. N^{15} incorporation into total plasma protein and albumin in a chronic uremic subject (YY) on intermittent hemodialysis on a diet containing minimal amounts of high quality protein.

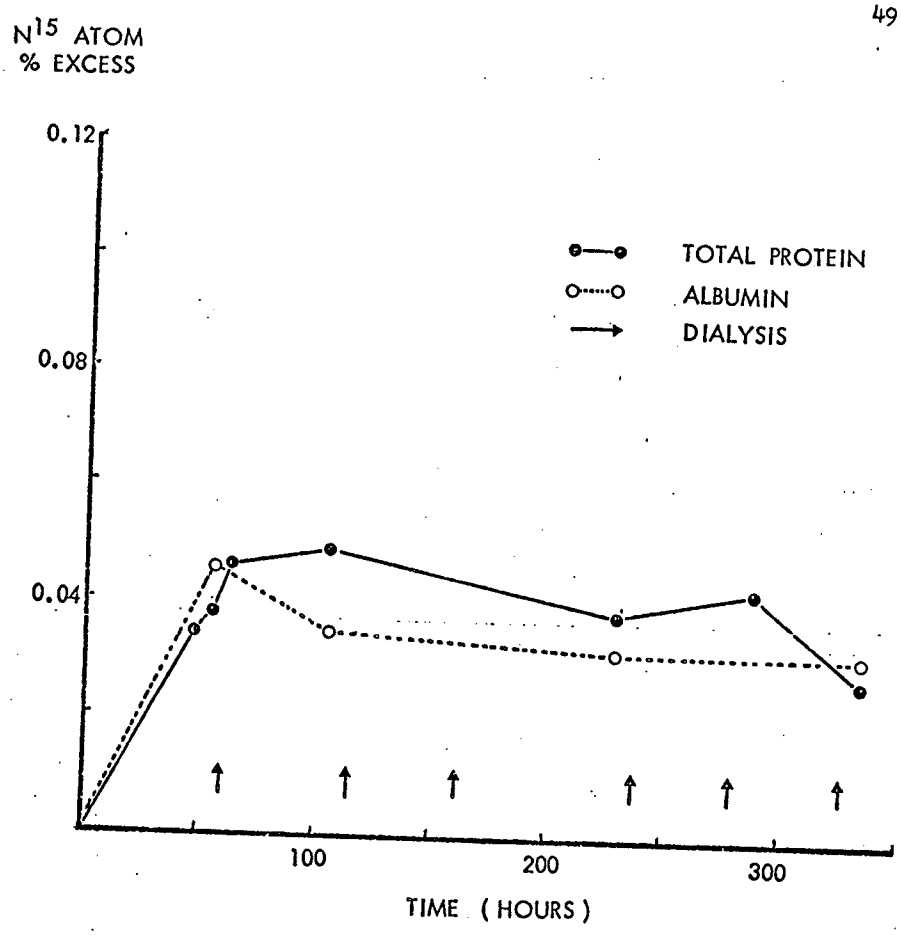


FIGURE 15. N^{15} incorporation into total plasma protein and albumin in a chronic uremic subject (CR) on intermittent hemodialysis on a diet containing minimal amounts of high quality protein.

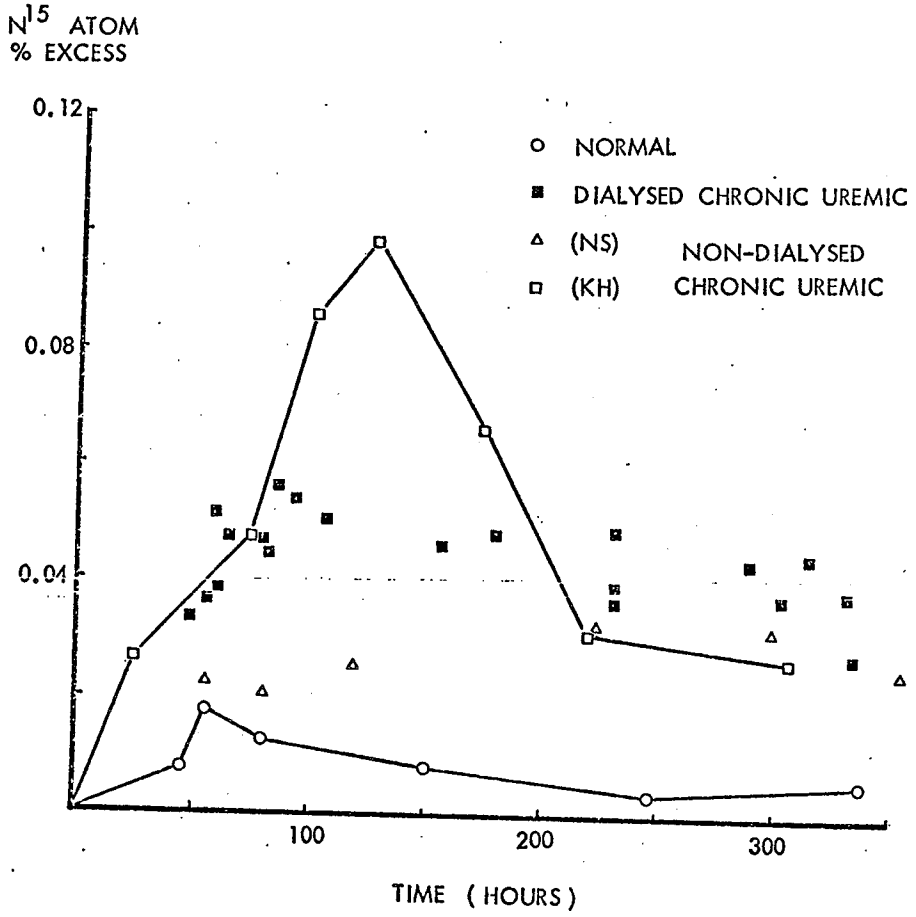


FIGURE 16. Comparison of N^{15} incorporation into total plasma protein in all subjects.

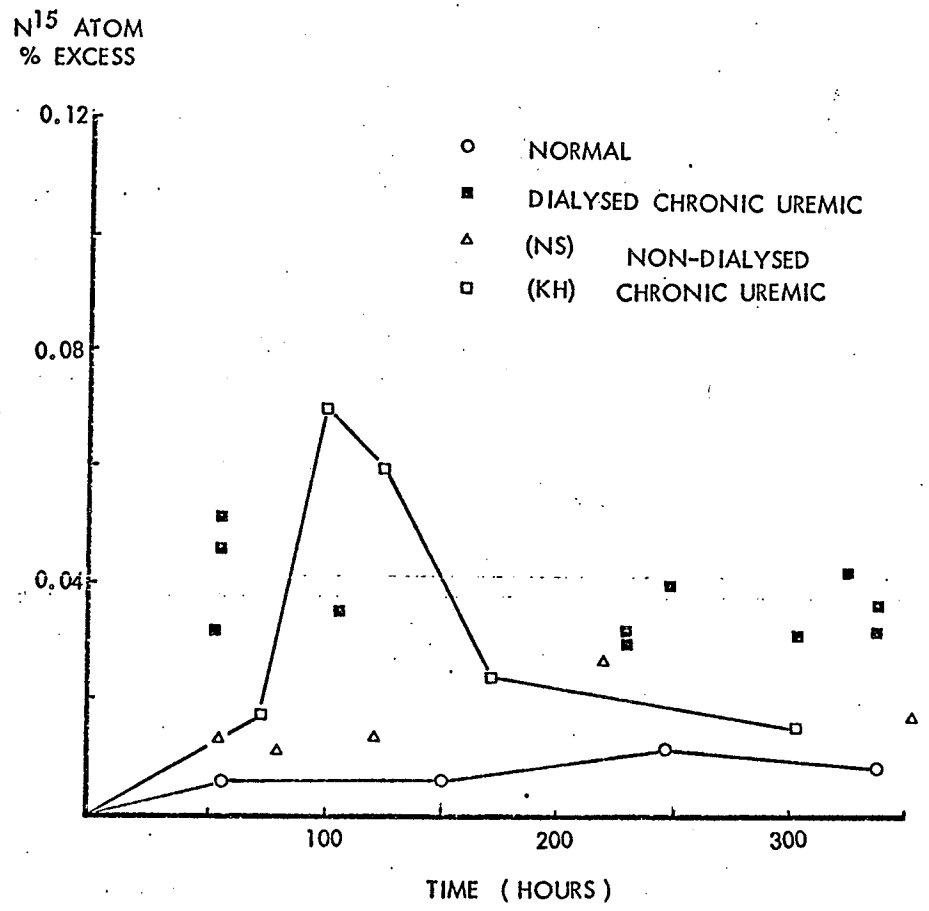


FIGURE 17. Comparison of N^{15} incorporation into serum albumin in all subjects.

Excess label persisted at high levels throughout the period of observation of some 340 hours in the four subjects who had the greatest, impairment of excretory function (Table II). Moreover, at the end of the two-week period there was a significantly higher level of isotope remaining in these patients than in the normal.

As shown in Table VII, the percent of administered dosage of N^{15} label appearing in the albumin was two to five-fold greater in the uremic individuals, the largest occurring in one of the non-dialyzed uremics.

TABLE VII. DATA USED IN CALCULATION OF N^{15} INCORPORATION INTO PLASMA ALBUMIN

Subject	B. Wt. kg	Max. Alb. Labelling % Excess N^{15}	Alb. Conc. g/100 g	N^{15} Dosage mg	Body S.A.+ sq m	Alb. Pool* N g	Max. N^{15} in Alb. Pool mg	Max. % of Dose Incorp.
I.G.	82.0	.011	4.8	1148	2.20	81.66	8.99	0.78
N.S.	48.5	.027	4.1	680	1.52	56.42	15.23	2.24
K.H.	64.1	.070	4.2	896	1.72	63.84	44.68	4.99
G.N.	63.1	.034	3.3	882	1.73	64.21	21.83	2.47
C.R.	61.4	.046	3.9	860	1.75	64.96	29.88	3.47
Y.Y.	63.5	.051	4.3	889	1.71	63.47	32.36	3.64

* Calculated from product of body surface area (sq. m) and factor of 232 (Sterling, 1951).

\+ Boothby, Berkson & Dunn. Nomogram for clinical application, Am. J. Physiol. 116:468, 1936.

DISCUSSION

The utilization of N^{15} from orally administered isotope in the normal subject on a protein restricted diet was in agreement with that found by other investigators.

Richards et al. (1967) studied N^{15} ammonia utilization in healthy subjects receiving unrestricted and restricted amounts of dietary protein. These workers elected to use N^{15} ammonia in preference to urea because there is evidence to indicate that the ammonia is the form in which the nitrogen from urea is utilized. This ammonia is formed by the bacterial breakdown of urea in the intestine. Their results revealed that the more severe the protein restriction, the greater the incorporation into plasma proteins in these normals.

Giordano et al. (1968b) showed that in the normal person, urea nitrogen is more easily utilized for amino acid synthesis when the dietary protein is very low. Read et al. (1969) could see no evidence of N^{15} utilization in the normal well nourished subject on an adequate protein diet.

It appears then, that the body must have some impetus to utilize non-protein nitrogen for protein synthesis.

It has been suggested that protein depletion might supply this impetus. The non-dialyzed uremic subjects of this study incorporated considerably more N^{15} into plasma proteins than did the normal (Table VI). Although not apparent clinically, these individuals were probably protein depleted. Giordano et al. (1968a)

felt there could be many factors leading to malnutrition in uremia. On examination of plasma aminograms of uremic patients he found that they resembled those reported by Snyderman et al. (1966) in states of protein malnutrition. Read et al. (1969) showed that children with kwashiorkor and marasmus incorporated N^{15} from ammonia and urea into blood cells and plasma proteins. Thus, it appears that the body is able to use non-protein nitrogen as a source of nitrogen for protein synthesis if the appropriate conditions are met, that is, depletion of protein stores or minimal amounts of dietary nitrogen supplied by the essential amino acids.

A striking difference in the incorporation of N^{15} may be observed in the two non-dialyzed uremics in this study. On examination of figures 11 and 12, a sharp peak is seen in the level of incorporation in subject K.H. at approximately 125 hours. There is a rather sharp decline following this peak. In subject N.S. a less pronounced and more delayed response in the plasma protein uptake of N^{15} is shown. This subject may, in fact, be atypical. Unfortunately, emergency surgery for appendicitis was necessary approximately two weeks prior to the institution of this study. In retrospect, it is probable that by the time the study began nitrogen equilibrium had not been achieved following the stress of this episode. During the period of study, BUN levels fell from 75 mg per 100 ml to 32 mg per 100 ml which could not be attributed to an improvement in renal

function as serum creatinine levels (a better indicator of renal function) rose slightly during this period. It is possible that N^{15} could have been diverted for use in wound healing during this period (Kinney, 1960).

We have thus been able to confirm previous work which showed that the non-dialyzed uremic is able to utilize N^{15} urea for anabolic purposes with simultaneous reductions in BUN levels, relief from uremic symptoms and a general improvement in well-being.

We studied the incorporation of N^{15} into blood proteins in the dialyzed uremic subjects in order to determine to to what extent this ability to utilize urea nitrogen for plasma protein synthesis was retained. If the presence of malnutrition in the uremic provides the stimulus for utilization of urea for protein synthesis, this ability may be lost as their nutritional status appears to be improved on the basis of their plasma aminograms (Giordano 1968a). These aminograms have been found to be normal with the exception of a decrease in tyrosine and an increase in 3-methyl histidine. This may have been due to increases in dietary protein from 0.3 g per kg in the non-dialyzed patients to 0.7 to 1.0 g per kg in the dialyzed patients.

The dialyzed patients in our study received approximately twice as much protein (of high biological value) as the non-dialyzed patients. This was necessary to compensate for free

amino acid and peptide losses during dialysis. Giordano (1967) emphasizes that because of these losses low nitrogen diets are contraindicated in patients who require dialysis.

An examination of Figures 13 to 15 indicates that these people have indeed utilized nitrogen from the administered isotope for protein synthesis. The maximum incorporation does not appear to be as great in these individuals as in the non-dialyzed uremics. This could be accounted for by the loss of nitrogenous substances bearing an N^{15} label during dialysis. Giordano (1968a) showed that free amino acids and peptides in amounts of 14 to 20 g could be lost in a six hour period of hemodialysis.

As a basis of comparison of the extent of isotopic enrichment in the two groups of uremic patients, the respective amounts of N^{15} incorporated in the albumin at peak levels were calculated. Use was made of the formula of Sterling (1951) who expressed the grams of exchangeable albumin pool as a product of surface area in square meters and the factor 232. As may be seen, (Table VII) the maximum per cent of dose taken up into the albumin pool for the dialysis subjects was less than in the non-dialyzed subject KH but significantly greater than in the normal.

A summary of biochemical data may be seen in Table VIII. BUN levels in the normal are seen to be somewhat lower while on the diet which is to be expected (Addis, 1947). There was no significant rise following administration of the isotope. This again is not surprising in light of Giordano's (1968b) report that there was almost complete recovery of administered isotope in the urine within 48 hours.

In the non-dialyzed uremics, subject N.S. showed a marked decline in BUN levels, falling from 75 mg per 100 ml to 32 mg per 100 ml. However, in subject K.H. an overall increase in BUN was noted. This may have been due to the fact that he had been on this same dietary regimen for some time (see preliminary study) and had been able to maintain a BUN at normal levels in spite of his reduced function. When the label was administered in the form of urea, he was unable to remove this increased urea load rapidly. BUN levels rose following administration then gradually declined reaching pre-isotope levels in four to five weeks.

It was more difficult to assess BUN changes in dialysis subjects as urea is removed at each dialysis. Changes which were noted included:

1) Subject G.N., while on the diet showed a pre-dialysis BUN less than the previous post-dialysis BUN. This would suggest that he was disposing of urea in some other fashion. Results of this study would appear to

TABLE VIII. SUMMARY OF BIOCHEMICAL DATA*

Subject	BUN mg/100 g		Plasma Protein g/100 g				P. Creatinine mg/100 g	
			Total		Albumin			
	Before Diet	After Diet	Before Diet	After Diet	Before Diet	After Diet	Before Diet	After Diet
I.G.	11	8	6.9	7.4	4.2	4.8	1.0	0.9
N.S.	75	32	6.9	6.7	3.9	4.1	8.9	9.2
G.N.	90	52	6.7	7.6	3.3	3.3	6.8	5.4
C.R.	45	16	6.8	6.7	3.1	3.9	11.4	11.6
Y.Y.	80	54	6.7	7.5	4.0	4.3	7.2	7.2
K.H.	19	26	7.1	7.1	4.3	4.2	6.1	6.8

* In case of dialysis patients values are pre-dialysis.

indicate that tissue synthesis was occurring.

2) Pre-dialysis BUN levels in subject Y.Y. were 20 to 30 mg per 100 ml lower than before institution of the diet.

3) Prior to the diet, subject C.R. had pre-dialysis BUN levels ranging around 45 to 55 mg per 100 ml, while on the diet his pre-dialysis levels fell markedly - ranging from 16 to 18 mg per 100 ml and falling on dialysis to 6 to 7 mg per 100 ml. Since the total protein intake had not been appreciably altered it can be concluded that the quality of protein played a significant role in these decreased levels.

Plasma creatinine and serum protein levels (total and albumin) did not change remarkably over the period of the study.

Richards et al. (1967) go so far as to envision a diet for uremia containing as the only source of nitrogen lysine and threonine. Ketoacids would replace all the other essential amino acids. This synthetic diet would contain carbohydrate, fat, vitamins and minerals to meet body requirements.

SUMMARY AND CONCLUSIONS

The use of a diet containing limited amounts of protein of high biological value was studied in normal and uremic subjects. Although it has been established that such dietary management can bring about biochemical and clinical improvement in the non-dialyzed uremic, it had not been established whether this ability was retained in the patient being maintained on intermittent hemodialysis.

The subjects selected for this study included a normal volunteer, two non-dialyzed uremics and three uremic subjects undergoing intermittent hemodialysis.

Incorporation of isotopic nitrogen into plasma protein was used as the criterion of non-protein nitrogen utilization for anabolic purposes. Following a one week stabilization period the isotope was administered and N^{15} incorporation followed at timed intervals for a two week period. The results indicated:

1) the biochemical status of the non-dialyzed uremics was improved, with associated amelioration of "uremic" symptoms.
2) the non-dialyzed uremics utilized significant amounts of non-protein nitrogen for plasma protein synthesis thus confirming the work of earlier investigators.
3) uremic patients being treated by intermittent hemodialysis demonstrated this ability to utilize non-protein nitrogen

but to a lesser extent than the non-dialyzed subjects. It was, however, significantly greater than in the normal subject.

These studies indicate that a diet containing 0.7 grams of high quality protein per kilogram of body weight would be a useful adjunct in the treatment of patients undergoing intermittent hemodialyses.

The chief limiting factor in the use of this diet appears to be the lack of acceptability. This can be attributed to the dearth of palatable commercially available low protein foods. It is hoped that the food scientist will combine his skills with those of the medical profession by contributing to the quality and variety of foods available for use by these patients.

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APPENDIX

APPENDIX A. Sample of four day diet cycle.

NAME: Cameron Robertson

DATE: Day #1

WARD:

BREAKFAST	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Grapefruit sections	150 g	0.7	1	202	---	15
Bread, toasted	200 g	---	500	20	10	110
Butter, S.F.	15 g	---	---	---	15	---
Marmalade	20 g	---	2	6	---	14
Egg, poached	one	6.4	61	65	5	---
Milk, whole	180 g	6.3	90	252	6	9
LUNCHEON						
Sandwich:						
Bread	120 g	---	300	12	6	66
Tomato	50 g	0.5	1	120	---	3
Butter, S.F.	10 g	---	---	---	10	---
Lettuce	20 g	---	---	35	---	---
Eggs, hard boiled	two	12.8	122	129	10	---
Apple, raw	200 g	---	1	220	---	28
Watermelon	200 g	1.0	2	200	---	11
Milk, whole	180 g	6.3	90	252	6	9

2/69.

NAME: Cameron Robertson

DATE: Day #1

WARD:

DINNER	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Mushrooms	70 g	1.8	11	390	---	3
fried in butter	10 g	---	---	---	10	---
Tomato, baked	70 g	0.8	3	170	---	4
Green beans, c'nd	50 g	0.7	---	43	---	3
Bread	160 g	---	400	16	8	88
Butter, S.F.	10 g	---	---	---	10	---
Cinnamon applesauce	150 g	---	---	97	---	34
Milk, whole	180 g	63	90	252	6	9
SNACK						
Bread, toasted	120 g	---	300	12	6	66
Butter, S.F.	10 g	---	---	---	10	---
Apple, raw	200 g	---	1	220	---	28
DAILY TOTAL		43.6	1975	2713	118	500
CALORIES - 3236						

2/69.

NAME: C.R.

DATE: Day #2

WARD:

BREAKFAST	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Grapefruit sections	150 g	0.7	1	202	---	15
Bread, toasted	200 g	---	500	20	10	110
Butter, S.F.	15 g	---	---	---	15	---
Marmalade	20 g	---	2	6	---	14
Egg, fried	one	6.4	61	65	---	---
in S.F. butter	10 g	---	---	---	10	---
Milk	180 g	6.3	90	252	6	9
LUNCHEON						
Egg, omelette	two	12.8	122	129	10	---
milk	30 g	1.1	15	42	1	1
Bread, toasted	120 g	---	300	12	6	66
Sl. tomato	50 g	0.5	1	120	---	3
Carrot curls	20 g	---	---	68	---	2
Butter, S.F.	10 g	---	---	---	10	---
Watermelon	200 g	1.0	2	200	---	11
Apple, raw	200 g	---	1	220	---	28
Milk, whole	150	5.2	75	210	5	8

NAME: C.R.

DATE: Day #2

WARD:

DINNER	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Casserole:						
Spaghetti	65 g	2.1	---	39	---	14
Tomato, S.F. c'nd	70 g	0.7	2	235	---	3
Mushrooms	20 g	0.5	3	83	---	1
fried in butter, S.F.	5 g	---	---	---	5	---
Bread cubes	20 g	---	50	2	1	11
dipped in butter, S.F.	5 g	---	---	---	5	---
Bread toasted	160 g	---	400	16	8	88
Butter, S.F.	10 g	---	---	---	10	---
Pineapple	150 g	---	1	144	---	28
Milk, whole	180 g	6.3	90	252	6	9
SNACK						
Bread, toasted	120 g	---	300	12	6	66
Butter, S.F.	10 g	---	---	---	10	---
Apple, raw	200 g	---	1	220	---	28
DAILY TOTAL		43.6	2017	2549	124	515
CALORIES - 3350						

2/69.

NAME: C.R.

DATE: Day #3

WARD:

BREAKFAST	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Grapefruit sections	150 g	0.7	1	202	---	15
Bread, toasted	200 g	---	500	20	10	110
Butter, S.F.	15 g	---	---	---	15	---
Marmalade	20 g	---	2	6	---	14
Egg, scrambled	one	6.4	61	65	5	---
with butter, S.F.	10 g	---	---	---	10	---
milk	30 g	1.1	15	42	1	1
Milk, whole	150 g	5.2	75	210	5	8
LUNCHEON						
Sandwich:						
Bread	120 g	---	300	12	6	66
Tomato	50 g	0.5	1	120	---	3
Butter, S.F.	10 g	---	---	---	10	---
Cucumber, raw	20 g	---	---	32	---	1
Eggs, hard boiled	two	12.8	122	129	10	---
Canteloupe	150 g	1.0	18	375	---	11
Milk, whole	180 g	6.3	90	252	6	9
Apple, raw	200 g	---	1	220	---	28

NAME: C.R.

DATE: Day #3

WARD:

DINNER	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Devilled mushrooms	50 g	1.8	7	207	---	2
fried in butter, S.F.	10 g	---	---	---	10	---
with broccoli	50 g	1.5	7	110	---	3
on bread, toasted	160 g	---	400	16	8	88
Butter, S.F.	10 g	---	---	---	10	---
Pears	150 g	---	1	120	---	30
Milk, whole	180 g	6.3	90	252	6	9
SNACK						
Bread, toasted	120 g	---	300	12	6	66
Butter, S.F.	10 g	---	---	---	10	---
Apple, raw	200 g	---	---	220	---	28
DAILY TOTAL		43.6	1991	2622	128	482
CALORIES - 3294						

2/69.

NAME: C.R.

DATE: Day #4

WARD:

BREAKFAST	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Grapefruit sections	150 g	0.7	1	202	---	15
Bread	200 g	---	500	20	10	110
Butter, S. F.	15 g	---	---	---	15	---
Marmalade	20 g	---	2	6	---	14
Egg	one	6.3	61	65	5	---
fried in butter, S.F.	10 g	---	---	---	10	---
Milk, whole	180 g	6.3	90	252	6	9
LUNCHEON						
Sandwich:						
Bread	120 g	---	300	12	6	66
Tomato	50 g	0.5	1	120	---	3
Butter, S.F.	10 g	---	---	---	10	---
Lettuce	20 g	---	---	35	---	---
Eggs, hard boiled	two	12.8	122	129	10	---
Canteloupe	150 g	1.0	18	375	---	11
Milk, whole	180 g	6.3	90	252	6	9
Apple, raw	200 g	---	1	220	---	28

NAME: C.R.

DATE: Day #4

WARD:

DINNER	AMT.	PROT.	Na / mgm	K / mgm	FAT	CHO
Asparagus, S.F. c'nd	60 g	1.5	2	100	---	2
on toast	160 g	---	400	16	8	88
with cheese sce, Parmesan	5 g	1.8	36	7	1	---
cornstarch	5 g	---	---	---	---	4
Butter, S.F.	10 g	---	---	---	10	---
Butter, S.F.	10 g	---	---	---	10	---
Peaches	150 g	---	2	196	---	30
Milk, whole	180 g	6.3	90	252	6	9
SNACK						
Bread, toasted	120 g	---	300	12	6	66
Butter, S.F.	10 g	---	---	---	10	---
Apple, raw	200 g	---	1	220	---	28
DAILY TOTAL		43.5	2017	2491	129	492
CALORIES - 3303						

2/69.

APPENDIX B. Sample of graph showing peaks 28 and 29 obtained from mass spectrometer.