ABSTRACT

EFFECT OF DIFFERENT SOURCES OF PROTEIN
ON SERUM UREA NITROGEN AND
ACID-BASE HOMEOSTASIS
OF HUMAN SUBJECTS

By

Mushtaq Ahmad

The entire study was divided into two parts, one being the effect of ingestion of sodium bicarbonate and the other being the effect of different sources of protein, i.e., animal, wheat and potato, fed isonitrogenously to human subjects and rats on the serum urea nitrogen (SUN) and acid-base homeostasis of human subjects in particular, and blood and urine composition in general.

Part I

Two experiments were carried out. In the first of these, three healthy, adult male human subjects consumed typical American diets of the same kind and same amount for five days.

They then ingested 15 g $NaHCO_3$ per day with the same diet for an additional five days. There was no variable except ingestion of bicarbonate between the two phases. During the bicarbonate phase, the urine pH became alkaline

and the total titratable acidity of the urine dropped from 22.6 ± 2.5 to 17.4 ± 4.5 mEq per day. The serum urea nitrogen also dropped from 16.7 ± 1.3 mg/dl to 12.7 ± 2.3 mg/dl ($P < .05$). There was no change in serum creatinine concentration. This suggests that the changes observed in acid-base balance have an effect on SUN concentration. Serum glucose and cholesterol levels were not changed by the ingestion of bicarbonate.

In the second experiment, during the control phase, four healthy adult male human subjects drank, for seven days, a constant volume of liquid diet in which wheat gluten was the only source of protein (Taylor _et al_. Brit. J. Nutr. 32: 407, 1974). After the seven day control phase, 10 g $NaHCO_3$ was ingested daily in three doses with the same diet.

When the control liquid diet was consumed by these subjects, they excreted acid urine with a pH of 5.6 and titratable acidity of 95.2 ± 30.3 mEq/day. When bicarbonate was ingested with the same diet, the urine pH increased to 7.3 and the titratable acidity dropped to 24.2 ± 5.3 mEq/day. At the same time, a possible reduction in SUN was observed in three of the four subjects, while an increase in SUN occurred in one subject. No change was observed in serum creatinine, sodium and potassium in either phase. This supports the observations made in the first experiment that the change in acid-base balance of human subjects, by ingesting $NaHCO_3$ has an effect on SUN.

Part II

To examine the effect of various kinds of proteins, two different plant protein sources, i.e., potato and wheat, were studied in three experiments with humans and one with rats.

The first study was designed to evaluate the effect of potato protein when it isonitrogenously replaced mutton. For that study 3 normal young men consumed typical American diets except that the protein intake was restricted to 46 g per day. During the experimental phase of the study, potato protein was incorporated into the menus so it isonitrogenously replaced the protein in the control diet.

The blood samples drawn at the end of the 7 days of the control period had SUN values of 13.0 mg/dl; the blood taken at the end of the 7 days of the experimental period had 7.0 mg/dl. This represented a significant reduction ($P < 0.05$) in SUN.

In the second experiment 6 healthy college students consumed diets in which practically all the protein came from mutton. After 7 days on that diet, potato protein replaced that in the mutton on an isonitrogenous basis. The blood drawn at the end of the control period contained 14.8 mg/dl of SUN which decreased to 10.3 mg/dl when the potato diet was eaten for 7 days. A slight increase in plasma CO_2 and HCO_3^- was also observed in the second experiment at the end of the experimental phase. The titratable acidity decreased from 20.7 ± 7.4 to 10.6 ± 1.8 mEq/day.

In the third experiment, two isonitrogenous rations containing 10% protein of either wheat or casein, were fed to two different groups of adult, male Sprague-Dawley rats. The rats were fed for four weeks ad libitum when blood samples were secured by cardiac puncture. Then the rats were pair-fed for another four weeks and blood samples were drawn at the end of that period by cardiac puncture. Serum analyses showed significantly ($P < 0.05$) lower urea nitrogen levels in the rats fed wheat rations than in those fed the casein ration, both during the ad libitum and pair-fed periods. The SUN values of 10% casein fed group were 13.9 mg/dl and 13.8 mg/dl on ad libitum and pair feeding respectively, while the 10% wheat protein fed group of rats had SUN values of 11.6 mg/dl and 11.5 mg/dl on ad libitum and pair feeding respectively.

Finally, in the fourth experiment, a group of eight healthy, adult, male, college students were selected for the experiment to demonstrate whether or not diets high in wheat (90% of the total daily protein intake) had an effect on the serum urea level as compared to a typical American diet which is omnivorous in nature. In order to check the consistency of the effect produced during the wheat diet on various parameters of fasting blood, period I and IV were repeated. For this purpose, four of the eight subjects agreed to continue for seven additional days when the "control" diet was repeated and for another six days when the strictly "bread" diet was served. The first control period lasted 15 days. Thereafter, for six days all eight subjects ingested 10 grams of sodium bicarbonate with their control diet to examine the alkalinizing effect on the serum urea nitrogen. Then for another two weeks, diets high in wheat were served.

During this experiment, a gradual reduction of serum urea nitrogen was observed. The SUN decreased in subjects when the bicarbonate was ingested together with the control diets. There was a further, but slight reduction in SUN when a rigidly monitored bread diet was served. There was no essential change in creatinine and uric acid concentrations of the serum. Throughout the entire period of study, the hemoglobin, total protein and albumin concentrations in the serum remained constant. Also, no change in the level of serum glucose was observed. Although urinary pH on control and bread diets was on the acidic side, the titratable acidity during the bread diet was significantly lower. The titratable acidity which was 34.0 ± 7.0 mEq/day on control diet dropped to 31.8 ± 2.9 mEq/day on wheat diet. This change in acid-base balance would suggest that the reduction in SUN in the subjects consuming wheat diets may be due to the change in acid-base balance as reflected by titratable acidity of the urine.

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INTRODUCTION

The prominent role played by plant proteins in the diets of a large segment of the world's population has led to increasing concern that these plant proteins, especially in wheat, are not of sufficient nutritional value for normal growth and development. This has been assumed true for both animals and human subjects. This question has been thoroughly reviewed by Vaghefi _et al_. (1) and Mickelsen (2). These authors conclude that the proteins in whole wheat and 70% extraction flour contain an adequate quota of all essential amino acids to maintain nitrogen equilibrium, if a sufficiently long adjustment period is used and if enough wheat product is consumed to meet the protein requirements of the subject.

A great deal of attention has been given to the nutritional quality of the protein in bread. The results of studies aimed at evaluating the protein in American white bread have been almost unanimous in concluding that it would not support growth when it was the only source of protein in a diet. There is no doubt about the low PER of bread as measured by the accepted rat bioassay. The primary reason for suspecting a disparity in results secured with the weanling rat and human subject; is the relative rates of growth.

The pioneer work of Bolourchi _et_ _al_. (3) left no doubt that protein in 72% extraction flour contains an adequate amount of essential amino acids to produce nitrogen equilibrium in healthy adult subjects. In that study which lasted 70 days, a full academic term, 12 young men were fed a typical American diet for three weeks. The protein in that diet was restricted to 70 grams per day. For the next 50 days, the subjects received a diet which also provided 70 grams of protein per day with 90 to 95% of it from 72% extraction flour. During that 50-day "bread" period, there was no animal protein in the diet. Nitrogen balances were performed using daily urine and stool samples.

These results indicated that during the first 10 days of the bread diet, all the subjects were in negative nitrogen balance. However, in the remaining 40 days of the bread diet period, the subjects were in positive nitrogen balance. The initial negative nitrogen balance during the first ten days of the bread diet has not been explained. It was probably due to a number of factors. The most obvious explanation is that during the negative nitrogen period, the requisite enzymes were being formed in concentrations adequate to cope with the new amino acid mixture presented by the bread diet. That this is of minor importance has been suggested by Bolourchi, _et_ _al_. (1938) who pointed out that the activity of most enzymes in the body exceeds, by a number of fold, the daily needs. The maximum increase in any one amino acid during the bread diet was only two fold that in the control period. To accomodate such a change should require but a day or so for the induction of the necessary enzyme activity even if its activity were inadequate initially. Other possible explanations may involve the effect that acid base changes in the body may have on enzymatic and physiological reactions. The over-all effects of the change in diet may also be important. It has been pointed out (Mickelsen, 1976) that the presence of large amounts of white bread in the diet produces alternations similar to those attributed to fiber.

There are several factors which limit the translation of animal protein bioassay results to human subjects. One of these involves the nutrient requirements. This is especially important for rapidly growing rats which require a high concentration of all nutrients in their ration. This requirement, which is far greater than that of human infants, is associated with the fact that the young rat grows relatively at a much faster rate than human infants (4). At birth, the rat weighs 5 to 6 grams; it is usually weaned on the 21st day, when it weighs 35 to 40 grams. That represents a seven-fold increase in body weight in three weeks. Under ordinary circumstances, the laboratory rat is started on nutritional experiments at weaning. Thereafter, it frequently increases its weight at the rate of 6 grams per day providing its ration is adequate. This means, it doubles its weaning weight in 6 days. On the other hand, the human baby gains weight at a much slower rate. Consequently, the dietary requirements, from a quantitative standpoint, of these two species are entirely different.

By limiting the protein level in a ration to 10%, many plant proteins produce poor growth in weanling rats, whereas animal proteins, so called "superior proteins," produce better growth per gram of protein and thus receive a high PER score. This was evident in Bolourchi et al.'s (3) studies where her weanling rats grew very poorly when fed a diet in which bread provided the only source of protein.

Another factor which has become more important as a result of the MSU bread study, is the time required to establish an equilibrium state in nitrogen balance studies. For nitrogen balance studies, many investigators working with adult human subjects have assumed and some still believe that three, five, or at the most, seven days are adequate for the development of an equilibrium state. Most workers claim that in such a period of time, it should be possible to determine the efficiency of a given protein; this is not true as has been shown in the Michigan State University bread study (1).

During that experiment, one of the unexpected observations made when the subjects ate the bread diet, was a marked reduction in blood urea levels (5). The first blood sample secured after the start of the bread diet was on the 25th day and the next on the 50th day. The average value at the latter time was 6.4 mg /100 ml , while at the end of the control period it had been 16.9 mg /100 ml.

There have been suggestions that the vegetarian diet and the acid-base equilibrium of the individual may have some impact on kidney function in general and reduction in blood urea levels, in particular. Most reports in the literature are either of a preliminary nature or limited in scope. Therefore, on the basis of the data available, it has been considered important to further investigate the effect of different dietary sources of protein, i.e., animal vs. vegetable, on renal function as well as blood and urine composition.

The nutritional status of patients with renal malfunctions have been the subject of study almost from the time of the first description of the uremic syndrome. The unique problem of deranged metabolism, precarious nitrogen balance, low protein intake, and states of malnutrition, have attracted nutritionists to the clinical environments of nephrology (6,7). The uremic syndrome is a constellation of clinical and physiological abnormalities resulting from extensive deterioration of renal function. It is believed that many of the manifestations of uremia, such as disturbed functions of the peripheral and central nervous systems, bleeding abnormalities, pericarditis, and gastrointestinal disturbances, are the results of retained toxic metabolites. The end-product of exogenous and endogenous nitrogen metabolism have long been suspected as the toxicants responsible for the uremic syndrome (8).

Urea, the major end-product of mammalian protein metabolism, when retained in the body, has been exonerated as the primary etiologic agent in the uremic syndrome.

During the past decade, advances in nutritional therapy along with the development of long-term hemodialysis and renal transplantion, have added immensely to the possibilities for treating chronic uremic patients. Anderson et al. (9) reported that of the three methods only nutritional therapy is applicable in every case.

The magnitude of the problem posed by chronic uremic patients is evident from the mortality statistics. Basing calculations on the report that only about one in five patients who die from this condition could benefit from dialysis (10). This represents 1500 patients per year in the United Kingdom and about 7500 patients per year in the United States. These figures are based on the reported yearly mortality of 7000 for Britain and 35,000 in the United States. In the United Kingdom, the estimated cost of treating one patient in a dialysis center is £2500 per year; in the United States, the cost is more than $3000.

To implement the recommendation of the Committee on Chronic Kidney Diseases in the U.S.A. for a national dialysis and transplantion program would involve the expenditure of one billion dollars from 1970-1975. The annual mortality rate in the United States is in excess of 30% for patients undergoing dialysis (10,11). Also, dialysis patients must spend many hours each week attached to a kidney machine.

These patients are under a severe dietary restriction and suffer important side effects that may include impotency, depletion of calcium in the bones, and neurological and psychiatric changes. On the other hand, yearly costs for transplant patients are close to $3,000 (12). From these figures, it may be seen that few countries have the wealth or medical resources to cope with all financial and logistical implications of dialysis and/or transplants.

Clements (13) suggested that the most important thing to remember about kidney transplants is not that they are marvels of surgical techniques, but that they are the outward and visible manifestations of the failure of medicine to preserve the integrity of this organ. It is for this reason that every effort should be made to determine how renal failure can be prevented or the patient maintained in reasonably good health for as long as possible before having to be subjected to either dialysis or a transplant.

The level of urea in the blood is used as an index of kidney function. Any unexplainable increase in the blood urea level represents a potential derangement in kidney activity (14).

One of the dietary factors that is associated with an increase in blood urea is the level of protein in the diet. The more protein that is consumed, the higher the level of urea in the blood. The concept that the only dietary factor affecting the blood urea level is the amount of protein in the diet was suggested by the work of a number of scientists

(15,16). Those investigators provided no basis for a conclusion other than that the level of protein in the diet was the only factor appearing to influence the blood urea level. Furthermore, earlier investigators (15,16) apparently had established the concept that the only dietary factor affecting the blood urea is the amount of protein consumed, since there was no theoretical reason for believing otherwise. Furthermore, no thought was given to the possibility that in normal individuals, the functioning of the kidney could be influenced by the nature of dietary protein.

That factors other than dietary protein may influence the blood urea level was demonstrated by some Edinburgh pediatricians (17,18). They reported that children who developed renal disturbances secondary to scarlet fever, showed a reduction in their high blood urea levels when they were given sodium bicarbonate. With that, most of the symptoms associated with renal disturbances disappeared.

The present concept of protein excretory products basically, is a confirmation of Folin's observations (19). He showed that in mammals, urea excretion is related to the dietary protein intake. That assumes that all proteins have the same effect on urinary urea excretion, and by indirection, the same effect on the blood urea level.

This concept dominated the attempts to provide dietary care for those patients required to undergo routine renal dialysis as a consequence of poor kidney function. To minimize the load imposed on the kidneys of these patients, their diets have been designed to provide as little protein as possible. By thus reducing the load on their kidneys, the patients should be enabled to extend the time between dialysis.

Admittedly, renal dialysis is only a temporary solution to the ultimate problem faced by these patients. Today, the long-term prognosis for these patients depends on the maintenance of a successful kidney transplant. Another potential approach, based only on theoretical consideration is, as soon as the abnormality has been discovered, to delay the development of the syndrome.

The rationale for this suggestion comes from the classical M.S.U. Bread Study. Additional support for this proposition is indirectly provided by the composition of the initial diets developed for renal dialysis patients. The Giovannetti and Giordano diets were based on wheat products. It is likely that wheat was the primary ingredient in these diets, since the Italians for whom they were developed, use a number of wheat products that are low in protein. This may have been the primary reason for the nature of these original diets.

One of the primary goals of the present study is to evaluate the effect of different proteins on the blood urea concentration of both human subjects and rats. The concept that is proposed as a justification for this project is that different proteins are not alike in-so-far as their effect on blood urea levels is involved. The results of this work should provide a firm basis for indicating the mechanism and possible implications of reduced blood urea levels brought about by a high bread diet.

For this purpose, the experiments were designed to test human subjects and rats to see whether blood urea levels can be changed either by (1) changing the nature of dietary protein, (2) changing the acid-base state of the individual, or (3) whether both factors work together to bring about this change.

PART I

REVIEW OF LITERATURE

The Influence of Diet on Renal Function in Health and Disease

The kidneys are paired on either side of the spine; they are flattened, bean shaped, and located retroperitoneally in the lombar region. In a 70 Kg. man, together they weigh about 300 grams and thus constitute 0.4% of the body weight. In spite of their small size, the kidneys hold the key position in controlling the homeostasis of the internal environment (20).

The kidneys act as an important metabolic and endocrine organ with profound effects on homeostasis. But, occasionally one of their vital function goes away. The structures, especially the proximal convoluted tubules, are also the guardians of the body's nutritional wealth.

Nutritional homeostasis can often be achieved by sound dietary and nutritional principles and practice even in advanced renal-urologic disorders.

The effect of diet on kidney function, size and composition, has thoroughly been reviewed in the Ph.D. thesis of Dr. Jenny T. Johnson, 1972 (21).

This review will be concerned primarily with the influence of quantity and quality of protein on a number of physiological effects--i.e., nature of protein on renal function, blood urea levels and acid-base homeostasis of the individual. Furthermore, the effect of change in the acid-base state of the individual on the blood urea nitrogen also will be reviewed.

Dietary Protein and Renal Function

Changes in the amount of dietary protein consumed substantially affect the function, metabolism and structure of mammalian kidneys. Several well documented and significant changes in renal concentrating ability (22-25) have appeared. These cover such topics as urea reabsorption, (26-30) glomerular filtration rate, renal plasma flow and maximal rate of tubular secretion of p-amino hippuric acid (31-34), activity of enzymes related to the intermediary metabolism of amino acids and carbohydrates (35-39) and growth and weight of the kidneys, and tubular structure (40,41).

Dicker *et al*. (42) have shown that in rats, a protein deficient diet led to decreased ability of the kidney to concentrate the urine. McCance and co-workers (43) evaluated the ability of the kidney to concentrate urine in Ugandan children on protein-deficient diets. When severely malnourished children were admitted to the hospital, the maximal urine concentration was less than half that attainable on discharge. The parents of these children also ate a low protein diet and could not produce a normally concentrated urine. When their diet was improved, they achieved the same concentration as their European counterparts. Also when Europeans ate the local protein-poor diet, their urinary concentrating ability decreased.

Likewise, semi-starved individuals have been reported to have an increased urine volume (44). In the Minnesota study, as an example, the urine volume was 3 to 4 liters in a 24 hour period, while the young men were starving. Although the subjects in the Minnesota starvation study had polyuria, no specific determination of renal function in these men was actually made. Pullman et al. (45) described experiments in which they fed normal adults diets high in protein (2.3 to 3 g protein per kg of body weight), medium and low in protein (0.1 to 0.4 g of protein per kg of body weight). Glomerular filtration rate and renal plasma flow dropped while the low protein diets were fed and increased with the high protein diets. Nielson and Bang (46) studied normal subjects fed varying amounts of protein and found that the glomerular filtration rate was relatively unaffected by changes in the protein content of the diet. Sargent and Johnson (47) reported that when normal subjects were fed a caloric-deficient diet, irrespective of the percentage of calories from protein, fat or carbohydrates, there was a constant reduction on glomerular filtration rate as measured by creatinine clearance. Over a period of four years, these investigators have studied a total of 211 subjects under strict control of diet, fluid intake and daily activity. They concluded that many alterations can be provoked by nutritional imbalance, caloric deficiency, dehydrateion, physical work, and extremes of temperature.

Table 1 summarizes data for renal plasma flow and glomerular filtration rate in children and adults with protein calorie malnutrition. Alleyne (48) found that the glomerular filtration rate (GFR) was markedly reduced in malnourished children and improved as they recovered. He could not find any consistent difference in inulin or PAH clearances between edematous and non-edamatous children. Gordillo _et al_. (49) measured inulin and PAH clearances in ten children who were at least 40% below average weights for their age. Seven of these children were well hydrated and three were severely dehydrated. In well-hydrated, malnourished children, the clearance rates were about half the normal values while in the dehydrated, malnourished they were about one-fifth normal. Working with adults, Klahr _et al_. (50) demonstrated a marked decrease in both GFR and the renal plasma flow (RPF) in ten adults with protein malnutrition. Values for these measurements increased following protein repletion. GFR increased somewhat during repletion leading to an increase in filtration fraction. On the other hand, no decreases were observed in the glomerular filtration rate and the renal plasma flow in five cases of severe malnutrition with protein "deficiency edema" (51). These subjects differed from others reported in the literature in that they were oliguric at the time of the experiment.

It seems that in children, protein calorie malnutrition leads to a decrease in both renal plasma flow and glomerular filtration rate (47,48). In adults, conflicting results on

TABLE 1.--Glomerular filtration rate and renal plasma flow in malnourished subjects.

	Malnourished				Repleted or Normal			
Investigator	No. of subjects	C_{in} ml/min	C_{PAH} ml/min	FF	No. of subjects	C_{in} ml/min	C_{PAH} ml/min	FF
Alleyne (48)	8 children	47.1	249.4	0.21	14 children	92.6	321.2	0.29
	7 children	42.9	184.0	0.27				
Arroyave *et al*. (25)	9 children	13.7	-	-	9 children	33.9	-	-
					17 normal children	45.0	-	-
Gordillo *et al*. (49)	10 children	23.0	108.4	-	24 normal children	64.0	294	0.23
Klahr (50)	10 adults	64.1	325.8	0.20	10 adults	88.3	381.1	0.24
McCance (43)	11 adults	119.4	-					
	11 adults	100.9	-					
Mollison, P.L.*	2 adults	53,80	230,383[b]					
	2 adults	124,141	340,710					

*Mollison, P.L. Observation on cases of starvation at Belsen. Brit. J. Med. 1:4, (1946).

C_{in} Inulin clearance

C_{PAH} Para Amino Hippuric acid clearance.

FF Fractional filtration, the ratio of glomerular filtration rate and total renal plasma flow.

[b]Diodone clearances.

the effect of protein malnutrition of GFR and RPF have been reported (47). These differences in adults may relate to the severity and duration of malnutrition.

The opposite situation, namely excessive protein intake has been reported to influence the kidneys. That was reported by Osborne and coworkers who found a marked hypertrophy of rat kidneys fed a ration containing high levels of casein (52). That this is not characteristic of all animal species was emphasized by Reid (53), who found no change in relative kidney weights even when guinea pigs were fed rations containing as much as 65% casein. That the increase in the size of the rats' kidney in response to an excessive dietary protein level may be associated with histological abnormalities was reported by Lalich (54). He observed glomerular degeneration in rats fed rations containing excessive protein levels.

That alterations in the functioning of the human kidney as a result of prolonged high protein intakes was suggested by Newburgh and colleagues (55). They had a subject who consumed 338 g of protein daily. Albuminuria occurred after 6 weeks and hyaline and granular casts were evident after 7 weeks on this regimen. The urine was normal after the subject was fed a high carbohydrate diet for days. Squier and Newburgh (56) showed that a high protein diet fed to adults is a renal irritant as evidenced in the appearance of red blood corpuscles in the urine of normal men; a high protein diet for a short time had no effect on blood pressure.

Blood Urea Nitrogen as an Index of Renal Function

Tests of renal function are of two types, those that determine the ability of the kidneys to dilute and concentrate the urine and those that measure the ability of the kidney to eliminate metabolic end products. Those of the first group are well established; those of the second group are not completely satisfactory. Besides the use of dye tests, or inulin, the measure of renal excretion is attempted by a determination of the amount of urea or non-protein nitrogen in the blood. In clinical work, blood urea estimations are usually carried out to determine whether or not there is an insufficiency in kidney function. It is obvious that the wide variation which exists in these normal values precludes such determinations from being an accurate measure of kidney function. Still, the very highest levels of blood urea concentrations are apparently only attainable when kidney elimination is defective.

Mosenthal and Bruger (57) in 1935, suggested the ratio of urea nitrogen to the non-protein nigrogen of the blood as an index of the effectively functioning nephrons, irrespective of blood urea level. Hannon (58), while summarizing his observations, reported that a steady fall in urea clearance indicates a steady decrease in the number of functioning glomeruli. Similarly, Peters and Van Slyke (59) suggested that the urea clearance is the most elegant method for estimating the ability of the kidney to eliminate this nitrogenous product since that is one of the most important renal functions. For this and other reasons, urea clearance has been widely used in clinical studies.

A high level of urea in the blood, although not toxic in itself, is a useful clinical index of the inability of the kidney to perform its function properly. As an easily measured index, it bears an important relationship to the functional reserves of the kidneys. In clinical work, blood urea estimations are usually carried out to determine whether there is an insufficiency in kidney function (60).

The normal plasma concentration of urea varies between 15 and 35 mg per 100 ml. If the clearance of this substance parallels the glomerular filtration rate, it is clear that when filtration rate falls the plasma concentration will rise. It would seem simpler therefore, when trying to gauge the state of GFR, to be guided by an estimation of these plasma concentrations instead of having to perform clearance tests. Unfortunately, the relation between glomerular filtration and plasma concentration is such that these concentrations are only of limited usefulness in this respect.

The reason for this is that the plasma concentration of urea depends on its rate of production, excretion, reabsorption and degradation. If its route of elimination is via glomerular filtration and its production is relatively constant, then a fall in GFR will cause the plasma concentration to rise and vice versa (61). On the other hand, a steady blood urea concentration of normal and uremics conceals a highly dynamic urea metabolism. Bacterial ureases in the colon continually hydrolyze urea to ammonia and carbon dioxide. Urea production, measured from the decay in plasma ^{14}C activity, exceeded urea degradation. Walser and Bodenlos (62) estimated that 25% of the total urea produced is hydrolyzed in the colon, and there is resynthesis of urea from ammonia liberated from urea in the colon and recycled to the liver in portal blood. These investigators confirmed their hypothesis by showing that when an antibiotic was given, the difference was reduced so that synthesis almost equalled excretion. A small difference remained as would be expected because their treatment would not completely have eliminated urease-producing organisms.

In the steady state, the rate of production of metabolic end-products like urea, must equal their rate of excretion and degradation. Since urea is the chief end-product of nitrogen metabolism in man, the production and excretion of urea depends on many factors which affect nitrogen metabolism. These include quantity and quality of protein intake, caloric balance, functions of the liver, and certain endocrine glands, and accelerated degradation of endogenous protein associated with trauma, infection, fever and breakdown of blood, tissue or the gastrointestinal tract. In as much as the plasma level of urea depends on the relation of urea production to urea excretion, it will change with alterations in any of these factors (63).

The clearance of urea is, or at least was the most widely used test of renal function. Its value depends on the fact that urea clearance is directly related to the glomerular filtration rate. As a guide to glomerular filtration, however, its main disadvantages are:

i) The clearance of urea is less than the rate of glomerular filtration.

ii) This discrepancy varies with the rate of urine flow.

iii) The procedure involved in a urea clearance, especially in a hospital ward, makes it very vulnerable to technical inaccuracies.

There is no technique by which glomerular filtration rate can be measured directly. All available methods depend on the estimation of the rate at which substances excreted by glomerular filtration are cleared from the plasma. If the clearance of a substance is to be an accurate index of the glomerular filtration rate, the substance must be filtered freely at the glomerulus, and neither reabsorbed, secreted nor metabolized by the renal tubule. This is why inulin has been used for that purpose.

Urea is freely filtered at the glomerulus, but a variable proportion of the filtered load of urea is reabsorbed by the renal tubule. Physiological studies indicate that the fraction of filtered urea which is reabsorbed is an inverse function of the rate of urine flow (64).

It is well known that the blood urea values increase beyond the normal range for a given level of protein intake, only when the glomerular filtration rate is reduced to 40-50 ml/min. If, on the other hand, a diet low in protein content is used, it is possible to achieve normal blood urea values in individuals with a glomerular filtration rate of about 5 ml/min.

Furthermore, if a patient with normal kidneys has a gastrointestinal hemmorhage, the blood proteins in the bowels are absorbed and this leads to an elevated urea level. In patients with minimal impairment of renal function in the nephrotic syndrome, the blood urea level is often elevated, if corticosteroids are being given simultaneously. Another cause for a disproportionately high blood urea is the combination of dehydration plus cellular breakdown seen in acute infection (65).

Blood Urea as Influenced by Protein Intake in Normal Subjects

Nigrogenous end-products excreted in the urine are to a large extent, the result of interaction between the diet and the organism. The nutritional status of the organism is a function of chronological and physiological age as well as dietary experience.

The concept of protein excretory products, basically, is a confirmation of Folin's observations that in mammals, urea excretion is related to the dietary protein intake. He reported as early as 1905, that with every decrease in the quantity of total nitrogen eliminated, there is a pronounced reduction in the per cent of that nitrogen represented by urea. Later on, many investigators confirmed the early suggestions of Folin (66).

That premise assumes that all proteins have the same effect on the urinary urea excretion and, by indirection, the same effect on blood urea nitrogen. This concept dominated the attempt to provide dietary care for those patients who are required to undergo routine renal dialysis as a consequence of poor kidney function. To minimize the load imposed on the kidneys of these patients, their diet was designated to provide as little protein as possible.

Nitrogen is a universal requirement of living organism. It is utilized as such in the form of atmospheric nitrogen or in various compounds by different species. Protein represents the most complete and complex nitrogen source. Amino acids are the simplest, complete nitrogen source for vertebrates and are the form in which nitrogen is ultimately utilized. Limitation of dietary protein in patients in not very advanced and complicated renal failure will lead to negative nitrogen balance and ultimately will aggravate the situation.

Normally, blood urea nitrogen levels are controlled by several factors. So far, the quantity of protein has been the only dietary aspect of this problem that has received any attention by investigators interested in this field of nutrition.

a. Quantity of Proteins

1. Humans

A relationship between the amount of dietary protein and the BUN level in human subjects, was reported by Addis and Wantabe as early as 1917 (67). These investigators observed an increase in the BUN levels of their four subjects when the daily protein intake was raised from 12 to 150 g or more. Thirty years later, the same group of investigators observed an almost doubling of BUN levels when the protein intake of 10 normal young medical students was increased from 0.5 g per kg of body weight to 1.5 (68). A further, but less dramatic increase in BUN levels, occurred when the protein intake was raised to 2.5 g per kg. MacKay and MacKay (69) stated that the BUN levels of normal medical students increased when their protein intakes were raised from 1.1 g per kg of body weight to 1.7 g.

Infants also appear to show an increased BUN whenever the protein intake is augmented (70). The latter work was done with different groups of infants. These studies showed that as the percentage of calories from protein increased from 7 to 10, the BUN increased progressively from 6.0 to 22.6 mg. per 100 ml. of blood.

Williams (71) studied 34 infants. These babies were fed varying amounts of protein, and their BUN levels were estimated on the 10th, 17th, 24th, and 31st days of life. He reported that in infants weighing more than 1500 grams, there was a statistically significant increase in the blood urea level after the periods when extra protein was given. When this extra protein was withdrawn, there was a statistically significant fall in the blood urea level. However, he did not observe any relationship between protein intake and BUN level in the infants weighing less than 1500 grams.

In another study, where 61 healthy infants, aged between 1 and 3 months, were studied (72), the infants were divided into three groups on the basis of their feeding history. Group A included breast-fed infants, Group B infants were fed a modified cow's milk formula and Group C were infants who had already been introduced to a number of commercially available solid foods, instant foods, and artificial milk formulae. The BUN level of breast-fed infants was significantly lower than that of the bottle-fed infants. Since bottle-fed infants have a much greater protein intake than those who are breast-fed, these investigators (71,72) concluded that the high urea values in healthy infants are the result of very high protein intakes. This, the investigators thought, might be of some concern since the ability of the immature kidney to withstand the stress of urea loading is limited.

Nichols and Danford (73) reported that the higher protein content formula fed as compared to low protein formula infants, resulted in a temporary rise in serum osmolarity, markedly elevated BUN, and increased urinary excretion of urea, calcium and phosphate.

On the other hand, Chitre *et al*. (74) reported that an increase in protein intake of medical students from about 40 g per day to 80 g. produced no change in the BUN levels. This seems quite contradictory to reports of several other investigators. One such report by Longley and Miller (75) indicated a progressive increase in fasting blood urea nitrogen when the dietary protein is increased from 0.3 to 2.4 g per kg of body weight, but no further increase with higher protein diets. Furthermore, they reported that progressive increases in blood urea nitrogen on high protein diets is apparently prevented by increased urine output, giving maximum clearance for a greater part of the 24 hours. Comparable observations have been reported by Nielson and Bang (76) who observed a reduction in blood urea when normal subjects were fed diets poor in protein.

2. Animal Studies

In mammals, the clearance of urea varies with the protein content of the diet. When the nitrogen intake is reduced, the urea clearance decreases, sometimes considerably, and when the nitrogen intake is increased, it can be increased to some extent. However, in animals, especially in dogs, variations in nitrogen intake have a greater effect on renal hemodynamics than in man. Factors affecting the urea excretion were thoroughly reviewed by Schmidt-Nielsen (77).

A protein-free diet fed to rats has markedly different effects on urea excretion and the level of enzymes involved in urea synthesis, depending upon whether or not a dietary source of energy is also provided (78). The level of urea cycle enzymes in the liver is not related to total protein content of the liver, but is a function of the rate of protein breakdown to urea (79). Indeed, as the level of protein in the diet of rats was increased, they had to breakdown more and more protein for their energy requirements, and urea synthesis and excretion increased. On a high protein, meat diet, the urea clearance in the dog was increased when compared with a low protein cracker meal or mixed diet (80). Several other investigators have published similar results relating urea clearance to the level of protein of the diet (81-83).

In a series of experiments, Shannon (84) found that the glomerular filtration rate decreased when dogs were fed a low protein diet; the reduction was in exact proportion to the decrease in urea clearance. Several authors (85) agree that the changes in urea clearance are directly proportional to the changes in the glomerular filtration rate and renal plasma flow. Smith (34) reviewed in detail the studies completed by Moustgaard (86) relating to the effects of protein on renal function in the dog which showed that after a high protein meal, the GFR and RPF of dogs were increased.

Glycine, when fed or infused, produced increases in GFR and RPF in the dog (87). Rats similarly treated showed no changes in GFR and RPF.

Osborne *et al*. (52) observed no changes of an inflammatory or degenerative nature in the kidneys of rats fed a 75% protein diet. Some minute tubular and glomerular changes occurred, but these were insignificant (39). Renal tubules were dilated throughout the kidneys of rats fed rations containing 70% or more protein. Moise and Smith (88) examined the remaining kidney of uninephrectomized rats fed 18% or 85% protein rations. No anatomic changes suggestive of significant renal damage in the 18% group were reported. The high protein group, on the other hand, showed significant glomerular and tubular changes.

In contrast, Addis *et al*. (89) reported no differences, upon microscopical examination, in kidneys of control and high protein- or high cystine-fed rats. Hogs fed a 42% crude protein diet had considerable renal damage when compared to controls fed a ration containing 13.6% protein (90). Similar reports relating the deleterious effects of high protein intake on kidneys were published by numerous researchers (91, 92).

There is good evidence that the blood urea level is influenced not only by renal or other diseases, but also, by the quality, quantity and proximity of the preceding meal. Street *et al*. (93) reported that the rise of blood urea nitrogen in beagle dogs fed four different diets was roughly proportional to the amount of protein consumed. The peak responses occurred 2 to 6 hours after feeding, and the elevations in the blood urea lasted about 9 hours in animals fed the low protein diet, and 21 hours in the dogs fed the high protein diets. A significant relationship between postprandial increase in blood urea and dietary sources of protein, was emphasized in the authors' report. Anderson and Edney (94) reported that the plasma urea levels in their dogs during the period of high protein intake were always greater than during the low protein intake. Furthermore, the postprandial values exceeded 40 mg. per 100 ml after the high protein meal, but never exceeded this level after the low protein meal. In their further studies, Street _et al_. (95) could produce a significant elevation in blood urea nitrogen which lasted 10-18 hours. It was true particularly, when beagles received the bulk of their daily protein intake at one meal.

It has been shown by Lewis (96) and Preston _et al_. (97) in ruminants, that dietary changes lead to different levels of blood urea concentration which can be correlated with different rumen-ammonia concentrations. Rabinowitz _et al_. (98) reported that the concentration of the plasma urea increased when sheep were changed from low protein to high protein diets. Similar results by a number of investigators have shown that the blood urea concentration in animals increases as the protein content of the diet increases (99-101).

b. Nature of Dietary Protein as it Affects Renal Function

Normal kidneys are well equipped to excrete urine at least as acid as pH 5; however, attempts, both medically and otherwise, to render body fluids alkaline are numerous.

With normally functioning kidneys, the body is able to maintain the pH of its fluids within a very narrow range despite the ingestion of large amounts of acidic or basic substances. As early as 1921, Haldene (102) showed that when normal subjects consumed 15 to 20 grams of ammonium chloride per day, acidosis could be produced. He was able to lower the bicarbonate level of his blood by one half. This amount of ammonium chloride (15-20 gm. per day) reduced the pH of the blood by 0.2 unit. The daily administration of 45 gm. of sodium bicarbonate was necessary to change the reaction of the blood to a similar extent in the opposite direction. To have an alkaline ash content equivalent to 40 gm. of sodium bicarbonate would require the ingestion of 18 pounds of oranges; to have an acid ash equivalent to 15 grams of ammonium chloride would require 4.5 pounds of lean beef or two pounds of oysters. This means that the kidneys have the capability of maintaining the blood pH within the narrow limits of 7.35 to 7.45 by producing urine as acid as pH 4.5. With that capability, normal kidneys can maintain the pH of body fluids within a narrow pH range despite the consumption of very abnormal diets which are likely to produce either a highly acidic or alkaline ash.

The use of a basic diet was recommended by Sansum and his associates (103) as early as 1923. These workers stated that even the slightest degree of acidity in the body is incompatible with life. We consume what food we please, and the body preserves its alkaline balance by secreting the excess acid formed in the metabolic reactions by means of the kidneys. They further stated that urine acidities 100 times greater than that of body fluids are very common, and, occasionally, a urine is found which is 1,000 or more times as acidic. These investigators observed the destructive action of very slight traces of acid on living tissue, and stated that abnormally acid forming diets may be a factor in the production of blood vessel and kidney diseases. Carter and Osman (104) advocated the treatment of nephritic patients by large doses of alkali, claiming that this disease is almost invariably associated with a lowering of carbon dioxide combining power of the blood and a highly acid urine.

It is common to find that a considerable diminution in the alkali reserves of the blood occurs in case of renal failure on an ordinary basis. The retained acids have to be neutralized by the body bases. It is not unreasonable to suggest, therefore, that the work of the diseased kidney may be considerably lightened by changing the nature of protein (i.e., from animal sources which are acid producing to a basic diet of plants).

One of the first indications that the nature of protein has an effect on blood urea nitrogen came from Lyon, et al. (18). In their study, only the kind of protein, and not the amount of protein, was varied; that change produced a marked drop in blood urea level. They suggested that the beneficial effects of vegetarian-type diets and the deleterious effects of high acid diets may be due to the nature of the amino acids in the different types of protein in addition to the basic reaction of vegetarian diets.

Since then, no report similar to that appeared, but there are indications that the type of protein does have an effect on the blood urea nitrogen. Levin's (105) formula diet for dialysis patients, consists of two or three choices of breads, vegetables, and fruits. Unique to this regimen was, as the author describes, the fact that dialysis was necessary only once a month.

Additional evidence that the nature of the protein influences the BUN levels was reported by Lange and Lenergan (106). They found a drastic drop in blood urea nitrogen after treating their patients, with an egg diet. After 30 months on the egg diet, a patient stated he could no longer live without meat; protein from beef and poultry was substituted for the eggs on a gram per gram basis. As a result, his blood urea nitrogen which had fallen by 62% shot back up to a level that was only one-third below pre-diet values.

In 1968, Bolourchi and co-workers (5) in the M.S.U. bread study, showed a marked fall of 50% in blood urea nitrogen when the nature of the dietary protein was changed. In their study, twelve healthy adults were fed a diet containing 70 grams of protein from the foods in a typical American diet for a 3 week control period. This was followed by the experimental phase of 7 weeks when the diets had no animal protein. About 90% of the protein in the diet during the experimental phase came from wheat products. When the nature of protein was changed, the blood urea dropped by over 50%. Similar observations have been reported by Kies and Fox (107) who found that when their subjects were shifted from normal diets to isonitrogenous wheat diets, the blood urea values dropped about 50%.

On the other hand, Eggum (108) investigated forty-two feeding stuffs of widely differing quality in nitrogen balance trials with rats. The results showed that there is an inverse relationship between the blood urea content and biological value of the diet. In the study, the author observed the lowest blood urea level when the egg diet was fed.

Eggum's conclusion appears to be confirmed by the work of Puchal _et al_. (109). They stated that when weanling pigs were fed isonitrogenous rations containing different proteins, the urea levels of the blood on the 26th day of the trial were inversely related to the weight gains of the animals. In their experiment, 42 pigs averaging 11.4 pounds of body weight and 22 days of age were fed for 28 days, five different rations providing 20 percent protein. The average weight gain of the group fed the dried skim milk ration was 20.9 pounds and that for the pigs fed the meat meal ration was 2.0 pounds. The urea levels ranged from an average of 13.4 mg per 100 ml of plasma for the pigs fed the dried skim milk ration to 33.1 mg per 100 ml for those fed the meat meal ration. These results suggested to the investigators an inverse relation between the biological value of protein and the BUN level in the animal consuming the protein.

There are a number of reports which support the relation between blood urea level and the type of dietary protein observed in the M.S.U. Bread Study. Nitsan and co-workers (110) reported that when all the protein in the ration was supplied by a high protein (21.5%) wheat flour, the blood urea in the adult rats was significantly lower, after 10 weeks, than in the rats fed a casein ration with the same level of protein. Phillip and Kenney (111) compared the BUN values of male West Africans with comparable Europeans living there. To the surprise of these investigators, low concentrations of BUN were consistent among the West Africans. The West African average BUN value were 13.9 mg per 100 ml as compared to 26.7 in a group of Europeans. Unfortunately, these workers did not report the data on protein intake of the subjects, their age, nutritional status, or urinary nitrogen.

Sterk et al. (112) examined the influence of protein sources in the rations on the blood urea level and weight gain in pigs. Contrary to Eggum's observations, these workers showed that blood urea concentration from the aspect of protein source was highest in the group of pigs fed the largest percentage of animal protein rather than plant proteins.

Kiriyama *et al.* (113) reported that the urea excretion of gluten-fed rats was significantly higher than that of the casein-fed group. Although nitrogen intakes were almost the same for both groups of the same age, the mg urea formed per rat liver was greater in the casein-fed group than the gluten-fed group. They showed further an increased activity of liver arginase in the casein-fed group. Taylor *et al.* (114), working with young adult men, examined the relationship between the concentration of serum urea nitrogen and the efficiency of dietary protein utilization. They observed a significant negative correlation between net protein utilization and urea levels.

In acute renal failure, the first rule has been to inhibit protein catabolism as far as possible, and conversely, to maximize anabolism. In some instances, a severely restricted protein diet or in some cases protein-free diets have been recommended. More recently several clinicians have described the deleterious effects of low protein diets that have been given to patients in chronic renal failure. Warde (115) reported that the imposition of a low protein intake for patients in chronic renal failure introduces in the long term more troubles than it is really worth. He stated that when consuming a low protein diet, a patient is obligated to eat a supernormal amount of carbohydrates and fat which will increase blood triglyceride levels and cause hyperlipidemia.

Similarly, Young and Parsons (116) have reported that all long-term dietary regimens which provide less than 40 gm of protein per day, despite a high caloric intake, are subject to the hazard, e.g. anemia, and suggested that they should be avoided. They further reported that a diet based on essential amino and keto acids are frequently nitrogen deficient, unappetizing, and, frequently, not available.

Relatively little work on the relationship of the nature of dietary protein to blood urea nitrogen has been done with animals and humans.

In a similar study, Barnicot and Sai (117) observed that West African male students who lived in London for an average of two and one half years, had a mean blood urea level of 24.8 mg %, while in comparable groups of English students the level was 28.0 mg %. However, blood secured from Nigerian University students in Ibadan contained 16.6 mg urea per 100 ml. It was reported that the Ibadan student ate meat daily, but most of them took beans several days a week, and some of them, eggs and milk occasionally. These were the only important sources of protein. Unfortunately, there were no data on the quantitative aspects of protein consumption.

Effect of Age on Blood Urea Levels and Glomerular Filtration Rate

For some aspects of renal function, the results obtained on adults and infants can be compared directly without reference to the weight of the kidney or the size of the body. Functions which may be expected to vary with size, such as the glomerular filtration rate and urea clearance, can be compared only if some basis can be found for eliminating the effects of size. McCance and Widdowson (118) indicated that most of the work on man has been carried out by medical personnel who generally have made their comparisons on the basis of surface area. Animal studies have been carried out mostly by physiologists and pharmacologists who have usually taken body weight as the basis for their comparison. They further reported that a new born baby weighs only one-twentieth as much as a man, but his surface area is one-eighth as great. If surface area is taken as the basis of comparison, the kidneys appear to be less efficient than if the comparison is made on the basis of body weight. All the bases of comparison, however, indicate that the glomerrular filtration rate in the newly born is very much lower than in an adult. McCance and Otley (119) demonstrated that new born rats have a very low urea clearance if compared with an adult on the basis of body weight, and it would be almost negligible on the basis of surface area; yet, blood urea is no higher after the first day or two of life than that of an adult. McCance and Strangeways (120) studied the renal function and general metabolism of normal full term infants under conditions of starvation and water restriction. They found that infants derived only 4% of their basal caloric requirements from protein breakdown, whereas, adults who had been subjected to similar degrees of starvation and hydropenia, relied upon their tissue proteins for some 17-18% of their basal calories. Some very abnormal serum urea values have been found by investigators from time to time in the new born infant. Snelling (121) observed in several babies, aged from one to four days who had been born after prolonged and difficult labour to have high values for the non-protein nitrogen in the serum.

Kennedy (122) demonstrated that overfeeding increases first, the size and then the number of cells in the kidneys of rats. A similar effect follows unilateral nephrectomy. They suggested and reported that the gradual loss of renal substance which is characteristic of aging, is analogous to partial nephrectomy, as far as its effects on surviving tissue are concerned. Oliver (128) sectioned the kidneys of 75 persons over the age of 70 who had shown no signs of renal disease during their life. In every case he found degenerative and atrophic changes, but these were always accompanied by arteriosclerosis. He suggested that renal aging was primarily an ischemic process. In subjects from 20 to 90 years of age, the glomerular filtration rate (GFR), the effective renal plasma flow (ERPF) and the excretory capacity for diodrast decreased 46, 53 and 44% respectively

(123-125). Shock (126) also observed a gradual diminution of the urea clearance and progressively increased blood urea nitrogen (BUN) with advancing age. Schwartz _et al_. (127), on the other hand, was able to show, in children from one to twenty years of age with normal renal functions, a linear relationship between age and plasma creatinine concentration. They also reported that the values of plasma creatinine in males were consistently higher than those for females at all ages above four years. These investigators could not demonstrate such a relationship as far as plasma urea concentration is concerned. They suggested that the urea level is dependent on non-renal influences. Although the figures from various sources shown in Table 2 are not in entire agreement, presumably because of differences in methods used for the urea estimations, they reveal the same tendency as that of non-protein nitrogen to rise during the first three days of life. Smith (128) reported that once an intake of milk sufficient to produce growth is provided, the blood urea of full term or premature infants is reduced to levels which are normal for adults (119). Auld _et al_. (129) found that when they gave glucose to a group of infants soon after birth, their catabolism decreased considerably as compared to starved infants. They suggested that early caloric feeding of infants is desirable.

A report which appeared (130) in the Russian literature showed the effect of carbohydrate and fat on urea metabolism in the ration of weaned piglets. The authors reported that

TABLE 2.--Distribution of urea concentration for boys and girls one to 20 years of age.

Age	Females			Males			p
	n	$\bar{x}$	s	n	$\bar{x}$	s	
1	8	4.91	1.23	9	4.82	1.71	0.9
2	13	6.23	2.47	18	4.93	2.12	0.2
3	24	5.08	1.29	30	5.07	1.58	0.9
4	28	4.57	2.02	49	4.78	1.40	0.6
5	44	4.68	1.36	50	5.52	1.74	0.02
6	44	4.81	1.63	62	5.23	1.56	0.2
7	50	4.67	1.39	58	5.44	1.74	0.02
8	61	5.02	1.61	60	4.84	1.69	0.6
9	61	5.16	1.84	52	5.60	2.68	0.4
10	46	4.67	1.82	58	5.55	3.00	0.1
11	57	4.51	1.62	56	5.04	1.73	0.1
12	54	4.23	1.18	67	5.18	1.46	0.001
13	41	4.82	1.71	53	5.24	1.65	0.3
14	30	5.38	2.18	44	5.11	1.90	0.7
15	22	4.87	2.11	40	5.35	1.62	0.4
16	16	4.77	1.59	24	5.18	1.48	0.5
17	12	4.56	1.64	12	5.67	1.59	0.1
18-20	15	5.41	1.46	19	5.48	1.26	0.9

n = Sample number; x = mean plasma urea concentration (mM/l); s = standard deviation; p = significance of difference between male and female means.

(Schwartz et al., (127))

Figure I. The three types of reactions giving rise to endogenous acid production. (Adapted from, Relman, A.S.(131))

ENDOGENOUS PRODUCTION OF ACID AND NET EXTERNAL ACID BALANCE

1. Oxidation of Organic Sulfur to SO_4

$$\begin{matrix}\text{Methionine}\\ \text{or}\\ \text{Cysteine}\end{matrix} \longrightarrow \text{Urea} + CO_2 + SO_4^{=} + 2H^+$$

2. Conversion of Neutral Foodstuffs to Organic Acids

a. $$\text{Glucose} \longrightarrow \begin{matrix}2\ \text{Lactate}^- & + & 2H^+\\ \text{Citrate}^{\equiv} & + & 3H^+\end{matrix}$$

b. $$\text{Triglycerides} \longrightarrow \text{Acetoacetate}^- + H^+$$

c. $$\text{Nucleoprotein} \longrightarrow \text{Urate}^{=} + 2H^+$$

3. Hydrolysis of Phosphoesters

$$R\ O\ \overset{\overset{O}{\|}}{\underset{\underset{O H}{|}}{P}}{-}O H \xrightarrow[\text{at pH 7.4}]{H_2O} ROH + \frac{0.8\ HPO_4^{=}}{0.2\ H_2PO_4^-} + 1.8\ H^+$$

$$R{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O H}{|}}{P}}{-}O{-}R \xrightarrow[\text{at pH 7.4}]{2H_2O} 2ROH + \frac{0.8\ HPO_4^{=}}{0.2\ H_2PO_4^-} + 1.8\ H^+$$

$$R{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O H}{|}}{P}}{-}O{-}\overset{\overset{O}{\|}}{\underset{\underset{O H}{|}}{P}}{-}OH \xrightarrow[\text{at pH 7.4}]{2H_2O} ROH + \frac{1.6\ HPO_4^{=}}{0.4\ H_2PO_4^-} + 3.6\ H^+$$

when corn or glucose was added to pig rations to provide energy, it decreased the activity of the urea cycle enzymes in the liver by 5 to 12%. They showed that the urea in the liver and blood and the excretion of urea in urine was also diminished; they further suggested that this resulted in an enhanced deposition of nitrogen in the body.

Acid-Base Disorders, Renal Function, and Blood Urea Nitrogen

It is important to thoroughly understand the role of the kidney in acid-base regulation before actually discussing the renal function in acid-base disorders.

The oxidation of neutral food stuffs inside the cell gives rise to endogenous fixed acids (131). The protons released by these acids react with bicarbonate in the extracellular fluid to yield the salt of the endogenous acid, plus H_2O and CO_2. Theoretically, there should be three general sources of endogenous fixed acids during the metabolism of neutral food stuffs, shown in the scheme on page 42 (Figure I). Although the buffer system of the body can easily accomodate a day's net production of H^+ by the metabolic reactions, prolonged survival would be impossible without a mechanism for excretion of H^+ and regeneration of the buffer. The two major control systems of the body are the respiratory and renal systems. Here only the renal regulatory system will be discussed.

The kidneys can excrete H^+ in several ways. First, they have the capacity to excrete free H^+. Actually, the renal tubule can increase the excretion of H^+ 1200 fold from 40 mEq per liter to 40,000 mEq per liter (as in urine with pH of 4.40). However, this takes care of a very small amount of the daily production (132). In health, tubular cells of the kidney are capable of producing NH_3 which then diffuses into the urine which accepts the protons and excretes it as an ammonium ion (133-135).

About 3,500 micro-Eq. per minute of bicarbonate is filtered through the glomeruli, but all, or nearly all, of this large amount is reabsorbed by the renal tubules. Filtered bicarbonate accepts a proton passively donated by hydroxylated CO_2 in the tubular cell in exchange for a sodium ion actively absorbed (136).

Acidosis, an increase in the hydrogen ion concentration of body fluids, can result only from retention of hydrogen ion or a loss of hydroxyl ion; retention of an ion such as phosphate, sulfate, or chloride cannot cause acidosis, although the two abnormalities frequently do exist. Increase in the hydrogen ion concentration in the plasma reduces the concentration of bicarbonate ions (136-137).

Metabolic acidosis develops sooner or later in most patients with chronic, progressive renal disease. Renal acidosis is the clinical condition of metabolic acidosis caused by impaired renal regulation of the body's ionic pattern. Renal acidosis is of two types, (1) "Renal tubular acidosis" refers to a relatively rare form of renal acidosis, which according to Relman *et al*. (138) occurs in a variety of renal tubular diseases in infants, children or adults. There is usually little or no azotemia and concentration of phosphate, sulfate, or organic acids are not increased; (2) The term "uremic acidosis" signifies the acidosis which develops in the end stage of any type of generalized renal failure. According to several other investigators (139-140) chronic, progressive renal disease usually results in some degree of systemic acidosis when the glomerular filtration rate is reduced to 25 ml./min.

According to Schwartz et al. (141) and Van Slyke et al. (142-144) the occurrance of acidosis is due to impaired reabsorption of bicarbonate and impaired production of ammonia. The reduction in plasma bicarbonate is usually accompanied by significant rises in phosphate, sulfate, and various organic acids.

Bricker et al. (145) and Slatopolsky et al. (146) have reported that these disturbances are largely due to the reduction in number of nephrons rather than of damage to the surviving nephrons. Elkinton (147) compared the degree of metabolic acidosis with the degree of azotemia in 46 patients with various types of renal diseases. The comparison was made with the CO_2 level of plasma and the blood urea nitrogen which were inversely proportional. Additional observations by this group (148-149) indicated that the plasma urea nitrogen was directly proportional to the acidity or inversely proportional to the carbon dioxide content of the serum. They reported that when values for serum CO_2 content are greater than 17 mM per liter, the serum urea nitrogen is greater than 40 mg per 100 ml. Similarly, if the CO_2 content of serum is between 12 and 17 mM per liter, then serum urea nitrogen is greater than 80 mg per 100 ml. and between 8 and 12 mM per liter, then the serum urea nitrogen is greater than 120 mg per 100 ml.

It seems that when acidosis is due to generalized renal failure, the severity of the acid-base disturbance is approximately proportional to the degree of azotemia (148-150).

Effect of Diet on Acid-Base Homeostasis, Renal Function and Blood Urea Nitrogen

Dr. E. V. McCollum in his book, A History of Nutrition (151) stated:

> von Bunge, in 1873, professor at the University in Dorpat, advanced the idea that Forster's animals perished sooner on the ash-free diets than did animals that were fasted. This von Bunge attributed to the accumulation of sulfuric acid in the body from the oxidation of the sulfur in the proteins fed.
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> At von Bunge's request, his pupil, N. Lunin attempted to test the validity of this theory. Lunin used adult mice, and fed them a diet composed of casein, cane sugar and water. The mice survived 11 to 21 days, whereas the control animals given only water died within 3 to 4 days. He tried adding enough $NaHCO_3$ to neutralize the sulfuric acid equivalent to the sulfur in the casein; this addition led to survival of mice for 12 to 30 days. When only NaCl, which has no neutralizing value, was added to the food, as accordingly he wrote in his dissertation, the mice survived for even shorter periods.

Another early observation was made by Claude Bernard (152) where he describes:

> One day, rabbits from the market were brought into my laboratory. They were put on the table where they urinated, and I happened to observe that their urine was clear and acidic. This fact struck me because rabbits, which are herbivora, generally have turbid and alkaline urine; while on the other hand, carnivora, as we know, have clear and acid urine. I assumed that they probably had not eaten for a long time, and they probably had been transformed by fasting into veritable carnivorous animals, living on their own blood . . . So I had rabbits fed on cold boiled beef (which they eat very nicely, when they are given nothing else). My expectation was again verified, and, as long as the animal diet was continued, the rabbits kept the clear and acid urine.

Whenever reaction of foods is mentioned, there is confusion over metabolic end-products versus gastric and intestinal products. The reaction of a food is frequently judged by the change in pH or titrable acidity of the urine produced by the ingestion of the food. However, there is no accepted amount of food (either on the weight or calorie basis) that should be consumed when tests are made of their acidity or alkalinity. Bodansky (153) reported that fruits and vegetables are base-forming and yield an alkaline urine. McClellan and DuBois (154) studied two normal men who volunteered to live solely on meat for one year. The total acidity of the urine during the meat diet was increased to 2 or 3 times that of the acidity on mixed diets and acetonuria was present throughout the period of the exclusive meat diet. But urine examinations, determinations of the nitrogenous constituents of the blood and kidney function tests showed no evidence of kidney damage. Bischoff _et al_. (155) found highly acidic urine when they fed their subjects an acid-ash or acid-forming diet or when the subjects were given one pound of steak every day. They could not observe any significant difference in the acid-base picture of a normal individual whose blood was drawn before breakfast, this was true whether or not the mixed diet contained excessively alkaline ash food or food stuffs. However, a diet containing a pound of steak temporarily lowered the plasma bicarbonate significantly in one of the four individuals studied. Blatherwick (156) also emphasized that the composition of the urine is markedly influenced by the characteristics of the food ingested. Foods which have a predominance of basic elements lead to the formation of a less acid urine. Conversely, foods with a predominance of acid-forming elements increase the urinary acidity. Potatoes, potato ash, oranges, raisins, apples, etc., are very effective in reducing the acid output.

Bridges and Mattice (157) also observed in their experiments that the urinary reaction responds very quickly to certain foods. Hunt (158) reported that by varying the dietary content of sulfur containing foods, the urinary output of acid can be made to increase or decrease in close correspondence with the output of sulfur. However, his data suggest that persons living on a diet with an alkaline ash do not produce a urine more alkaline than their plasma. Furthermore, he showed a correlation between the mean daily output of calcium and the mean daily output of titratable acid (H^+). The suggestion made by Hunt that it is chiefly the oxidation of sulfur that determines the urinary excretion of acid, prompted Lemann and Relman (159) to investigate further the relationship of sulfur metabolism to acid-base balance. They observed in their experiment with human subjects that the administration of methionine produced small reductions in serum carbon dioxide content, associated with acidification of urine and increased excretion of ammonia. Another element that may influence the acidity of the urine is P. Lennon et al. (160) studied acid production associated with the metabolism of foods containing organic phosphorus. They found that acid production depends upon the chemical form of the phosphate residues (see Fig. I, p. 42). These investigators fed large supplements of purified soy protein and of beef steak to normal subjects and compared the effects on the acid balance. They reported that the soy protein generated 3.9 mEq of acid per gram of nitrogen and the beef steak, 2.9 mEq per g of nitrogen. It is suggested that the production of acid associated with the presence of phosphate group in a protein will be determined by the structural relationships of the phosphate and the nature of the associations. Several additional reports appeared by these researchers indicating that diet does affect the acid-base state of the individual (161-162).

It was in 1931, that a paper by Lyon et al. (17) indicated that children who developed a kidney infection associated with scarlet fever were markedly improved as far as renal involvement was concerned, when $NaHCO_3$ was administered. They reported that alkaline treatment usually produced clinical improvement, and they further described that coincidently there was a reduction in blood urea nitrogen with an increase in alkali reserve, and sometimes, a distinct diminution in the output of urinary albumin. In another report, Lyon et al. (18), described the beneficial effects of alkalis and the deleterious effects of acids on kidneys. They mentioned in their report that Bright himself advocated the use of an alkaline mixture in the treatment of the disease which bears his name. Further, they reported that in 1888, Von Jaksch and in 1912, Straub and Schlayer, noted a diminution in the alkalinity of blood in uremic patients, and it has since been observed by Sellards (163) that nephritics require a larger dose of sodium bicarbonate by mouth, to render their urine alkaline than do normal individuals. In those days, this observation had been made on the basis of a renal function test. Palmer and Henderson (164) have made similar observations. Since then, it has been shown experimentally by several investigators, that herbivores with a normally alkaline urine gradually showed considerable quantities of albumin in their urine when fed highly acidic diets of liver and oats (165-166). Coincidentally, with this manifestation, their blood urea and blood pressure rose and the alkali reserve of their blood, as measured by its carbon dioxide combining power, decreased. The animals eventually died from uremia unless green food was added to the diet. That the reverse of this change does not produce any abnormalities in carnivores as represented by the dog was demonstrated by Sansum et al. (166). They fed dogs a diet containing the same amount of soy protein, as used by Newburgh et al. (165), but in the form of soya beans. According to Sansum and co-workers, the diet was highly basic and produced an alkaline urine. Despite that, Sansum et al. (166) also demonstrated similar observations when a group of experimental animals were fed a diet containing an exactly similar quantity of protein, but consisting chiefly of soya beans, which according to the investigator, rich in protein, was of a highly basic character and giving an alkaline urine. These animals did not exhibit the nephritic phenomenon and remained in good health in spite of their high protein diets.

Elkinton et al. (167) reported that irrespective of cause, chronic progesssive renal disease usually results in some degree of systemic acidosis when the glomerular filtration rate is reduced to 25 ml/min or less. The occurrence of acidosis under these circumstances has been attributed to two interrelated disturbances of the renal acidification process: (1) impaired reabsorption of bicarbonate and (2) impaired production of ammonia. The reduction in plasma bicarbonate, usually accompanied by significant rises in phosphates and sulfates, has been reported by Relman (131) in uremic acidosis. As early as 1923, Marrack (150) reported:

> On ordinary diet, as a result of oxidation of neutral sulfur and phosphorous in the food to form sulfuric and phosphoric acids, and of the formation of organic acids, an excess of non-volatile acids arises daily, equivalent to about 500 c.c. of N/10 solutions, which should be excreted by the kidneys. When the kidneys are inadequate for this task, retention of acid occurs, just as retention of urea occurs when the kidneys are unable to excrete urea as fast as it is formed. Because as a general rule, on ordinary diets, the kidneys are presented with an excess of acid to excrete, alkali deficit is more common than alkali excess in untreated cases of nephritis."

Furthermore, the investigator demonstrated the incidence of mild, moderate and severe alkali deficit in 216 patients who had not been treated with alkalies. He then arranged them according to the height of the blood urea; when that was done, the patients' acidity was correlated directly with their BUN levels (150).

Several other investigators have shown an inverse correlation between alkalinity of the blood and urea levels of the blood (147-148). In many patients with chronic azotemic renal failure, renal reabsorption of bicarbonate is reduced (147,168,169). This abnormality was first recognized by Schwartz _et al_. (170); substantial amounts of bicarbonate appeared in the urine of five of twelve patients with chronic renal failure who were studied after their acidosis had been corrected with alkali therapy. Furthermore, these investigators reported the cause of the impairment of renal bicarbonate reabsorption in renal failure as being due to the fact that chronic renal disease reduces the activity of renal carbonic anhydrase. However, they regarded the evidence in support of this possibility as unconvincing. Henderson and Palmer (171) found that patients with chronic nephritis, most of whom were clinically uremic, and probably acidotic, excreted urine of high acidity and lower pH than normal. These workers reported a reduction in the excretion of ammonia, subsequently confirmed by Hartman and Darrow (172). Van Slyke et al. (173) observed that this reduction in NH_3 excretion was roughly proportional to the decrease in urea clearance. Later studies by Levinsky (174) are in agreement with these early observations. Wrong and Davies (136) also found that the excretion of titratable acid was significantly reduced, owing to a decrease in the excretion of phosphate buffers. On the other hand, David et al. (175) have suggested that any patient with renal failure should receive the maximum amount of fluid per 24 hours that his kidneys can excrete without developing evidence of congestive edema. The reason for this, as explained by these workers, is that urea, and possibly other toxic metabolic products, back diffuse into the blood throughout the length of the tubule. An increase in urine flow through the tubule should increase the amount of these products excreted since they would have less time to back diffuse.

Whang and Reyes (176) demonstrated that, following bilateral nephrectomy in male, adult Sprague-Dawley rats, correction of metabolic acidosis by the administration of $NaHCO_3$ resulted in significant amelioration of the hyperkalemia. These investigators observed azotemia in all nephrectomized groups. However, amongst the anephric group, the blood urea nitrogen was significantly lower in those animals receiving $NaHCO_3$. Similar observations have been made by Van Assendelft and Mees (177) who carried out balance studies on 21 patients with chronic renal insufficiency. They wanted to study the influence of the administration of $NaHCO_3$ and sodium chloride on urea metabolism. After a suitable control period, $NaHCO_3$ was given until a new steady state was reached. In every patient but one, plasma urea level fell. They further reported that this appeared to be the result of increased urea clearance, as well as decreased urea production.

In summary, it seems clear that urea metabolism of the human subjects and animals as well, is influenced by a change in acid-base homeostasis of the individual. The urea level of the blood appears to be low when alkaline conditions are produced in the body as reflected by urinary pH and/or titratable acidity.

PART II

EFFECT OF SODIUM BICARBONATE INGESTION ON SUN

Chapter I

Effect Of Ingestion Of Sodium Bicarbonate On SUN Of Normal Individuals

Introduction

It was in 1931 that it was shown that the blood urea level might be influenced by the acid-base condition of the body. That possibility was suggested by the work of Lyon et al. (17,18) who described BUN changes in patients with kidney malfunction and severe acidosis. When these patients were given sodium bicarbonate, not only was their acidosis corrected, but their elevated blood urea levels were reduced to practically normal and, with that, most of the symptoms associated with the renal disturbances disappeared.

On the basis of Lyon's observations, it was postulated that the blood urea nitrogen in normal subjects can be changed by altering the acid-base condition of the body. Since most American diets contain fairly large amounts of protein from meat, the utilization of which is associated with an acid production, it is likely that individuals on meat diets tend to have acidosis in contrast to individuals on vegetarian diets who tend to have alkalosis.

To further test the hypothesis that alkalosis lowers SUN, this study was undertaken to determine the effect on serum urea nitrogen that an alkalinizing agent, $NaHCO_3$, would have when there was no other dietary change.

Experimental Procedure

Subjects

Three normal adult subjects, free of gross signs of thyroid, neurological, muscular and renal disease were selected for the study.

Schedule

The study consisted of two phases; control phase, which was followed immediately by the experimental phase. Each of the dietary periods (control and experimental) consisted of five days. Twenty-four hour urine samples were collected on alternate days throughout the entire period of the study. Blood samples were drawn at the end of each dietary period, (i.e., both control phase and experimental phase).

Procedure

a. Control Period: The menu was similar to that routinely followed by the subjects prior to the initiation of the study. The menu consisted of either weighed chicken or weighed hamburger as the main source of protein. Other ingredients in the diet were maintained constant. They included lettuce, carrots, cauliflower, 6 oz of orange or tomato juice per day, and six slices of white bread with butter and jam every day. The food served was exactly the same in quantity and quality throughout the entire period of study.

b. Experimental Period: The dietary intake of protein, carbohydrates, fats, minerals, etc. was exactly the same as during the control period because the foods ingested were the same. On the morning of the sixth day from the start of the experiment, (i.e., at the end of the control period), 5 grams of $NaHCO_3$ was ingested in 25 ml of water. This supplement was consumed with breakfast, and again at lunch, and dinner to provide a total intake of 15 grams of $NaHCO_3$ per day. The supplement was continued throughout the experimental period. Although water intake was not specifically measured, an attempt was made to maintain daily water intake constant. However, it should be noted that urine flow rate increased at least 20% by the end of the experimental period in all three subjects; thus water intake may not have been accurately maintained constant between the control and experimental periods.

C. Sample Collection

1. Urine

Twenty-four hour urines were collected in half-gallon, wide-mouth, polyethylene bottles. About 15 ml of toluene was used as a preservative. The subjects voided and discarded the urine sample on arising on the first day of the study. On each bottle the name of the subject and the time in hours and minutes was recorded, so that 24 hour samples were collected with less than 30 minutes error.

2. Blood

Before breakfast, all subjects reported to Olin Health Center, Michigan State Univ., on the first day of the control and experimental periods. The latter represented the end of the control phase. Blood samples were secured at the end of the experimental period. Blood samples were drawn from each subject into a test tube from the anticubital vein. The samples were kept in a refrigerator for 4-6 hours and then centrifuged for 10 minutes. Serum was obtained with a pasteur pipette and was preserved in a freezer until analyzed for urea, creatinine, cholesterol, and glucose. Since no inhibitor of glucose metabolism was added to the blood when it was collected, the serum glucose analysis was probably an underestimate.

Analytical Procedures

A. Urine

As soon as the urine samples were brought into the laboratory, the total volume was recorded for each subject. Approximately 20 ml. of the sample was taken into a small glass beaker for the determination of pH with a Fisher Model 420 pH meter.

The titratable acidity of the urine was determined by the Folin method (178). 25 ml. of the urine in a 200 ml. flask to which 5 grams of finely pulverized potassium oxalate and few drops of 1% phenolphathaline was added. The contents of the flask were titrated with 0.1N sodium hydroxide until a faint pink color appeared. The total mEq of base required to titrate the 24 hour urine samples was calculated for each subject.

B. Blood

The frozen serum samples were sent to the commercial laboratory, Laboratory of Clinical Medicine, Lansing, MI., where they were analyzed by SMA - 12/30 autoanalyzer (179), for urea, creatinine, glucose, and cholesterol.

Statistical Analysis

The Wilcoxan's rank sum test (180) and paired t-test were used to assess the significance of the difference between the control and treated periods. The Wilcoxan test is more valid than student "T" tests when data is coming from non-parametric distribution.

Results

Serum Urea Nitrogen (SUN)

Three normal subjects were maintained on their normal omnivorous diet for 5 days. The blood urea nitrogen at the end of the fifth day (16.7 ± 1.2 mg/dl) was the same as at the start of the study (17.2 ± 1.3 mg/dl). After the end of the experimental phase, during which each subject consumed 15 grams of $NaHCO_3$, the serum samples for each of the three subjects, had lower urea nitrogen than that secured prior to the bicarbonate consumption (Table 3). There is a statistically significant reduction in SUN. The SUN values dropped from 16.7 ± 1.2 mg/dl on the control diet to 12.7 ± 2.3 mg/dl on the experimental diet.

Serum Creatinine

The average concentration of creatinine in the three subjects was the same at the end of the experimental period (0.6 ± 0.2 mg/dl) as it was at the end of the control period (0.7 ± .1 mg/dl).

TABLE 3.--Effect of the ingestion of 15 grams $NaHCO_3$ per day in three equal doses on serum level of urea and creatinine in three normal subjects who were (1) on typical American diet for five days as control and (2) same amount and same kind of diet + 15 gms of $NaHCO_3$ per day in three equal doses for five days. Blood samples were taken at the end of each dietary period.

Subjects	CONTROL		EXPERIMENTAL	
	Urea Nitrogen mg/dl	Creatinine mg/dl	Urea Nitrogen mg/dl	Creatinine mg/dl
C.M.	17.5	0.4	10.1	0.6
O.M.	15.2	0.8	13.5	0.8
M.A.	17.5	0.6	14.5	0.6
Average	16.7 ± 1.2^{x}	0.6 ± 0.2	12.7 ± 2.3	0.7 ± 0.1

SUN values during experimental period are significantly lower than control period ($P<0.05$).

^{x}Standard deviations are given for each average value.

TABLE 4.--Effect of the ingestion of 15 grams $NaHCO_3$ per day in three equal doses on urinary acidity in three normal subjects who were (1) on a typical American diet for five days as control and (2) same amount and same kind of diet + 15 grams of $NaHCO_3$ per day in three equal doses for five days.

Subjects	CONTROL typical American diet			EXPERIMENTAL typical American diet + 15 grams of $NaHCO_3$		
	24 Hour Urine Volume ml	Urinary pH	Titratable Acid Excretion mEq/day	24 Hour Urine Volume ml	Urinary pH	Titratable Acid Excretion mEq/day
C.M.	1348	6.03	25.6	1608	7.80	20.4
O.M.	1185	5.49	20.8	1382	7.71	12.2
M.A.	1136	6.50	21.5	1594	7.81	19.6
Average	1223 ± 110^{x}	6.00 ± 0.50	22.6 ± 2.6	1528 ± 126	7.70 ± 0.50^{xx}	17.4 ± 4.5^{xxx}

^{x}Standard deviations are given for all average values.

^{xx}Urinary pH is significantly higher ($P<0.05$) at the end of experimental period.

^{xxx}Titratable acid excretion per day is significantly lower ($P<0.05$).

TABLE 5.--Effect of the ingestion of 15 grams $NaHCO_3$ per day in three equal doses on

TABLE 5.--Effect of the ingestion of 15 grams $NaHCO_3$ per day in three equal doses on serum level of glucose and cholesterol in three normal subjects who were (1) on typical American diet for five days as control and (2) same amount and same kind of diet + 15 gms of $NaHCO_3$ per day in three equal doses for five days.

	CONTROL		EXPERIMENTAL	
Subjects	Glucose mg/dl	Cholesterol mg/dl	Glucose mg/dl	Cholesterol mg/dl
C.M.	82	200	85	200
O.M.	87	180	95	170
M.A.	80	190	85	210
Average	83 ± 3^x	190 ± 10	88 ± 6	193 ± 2

^{x}Standard deviations are given for each average value.

Urinary pH and Titratable Acid Excretion

There was an increase in the alkalinity of the 24 hour urine sample when sodium bicarbonate was ingested since the pH of the urine increased markedly from 6.0 to 7.7 (Table 4). The titratable acidity dropped significantly (Table 4), from 22.6 ± 26 mEq/day to 17.4 ±4.5 mEq/day (Fig. 2).

Although the fluid intake was kept as constant as possible, the urinary output increased in the experimental phase.

Serum Glucose and Cholesterol

These two parameters of the blood were practically the same at the end of the experimental period as at the end of the control period (Table 5).

Discussion

These studies confirmed those of Lyon *et al.* (17) described in the introduction to this chapter and thus the same conclusion reached by Lyon *et al.* also apply to this study. In our studies with normal subjects the addition of sodium bicarbonate to the typical American diet resulted in a low SUN. The cause of this decrease in SUN was either (1) decreased urea production, (2) increased urea clearance by increased GFR and/or increased urine flow, or (3) dilution of the total body urea by the addition of extracellular fluid to body with ingestion of sodium bicarbonate.

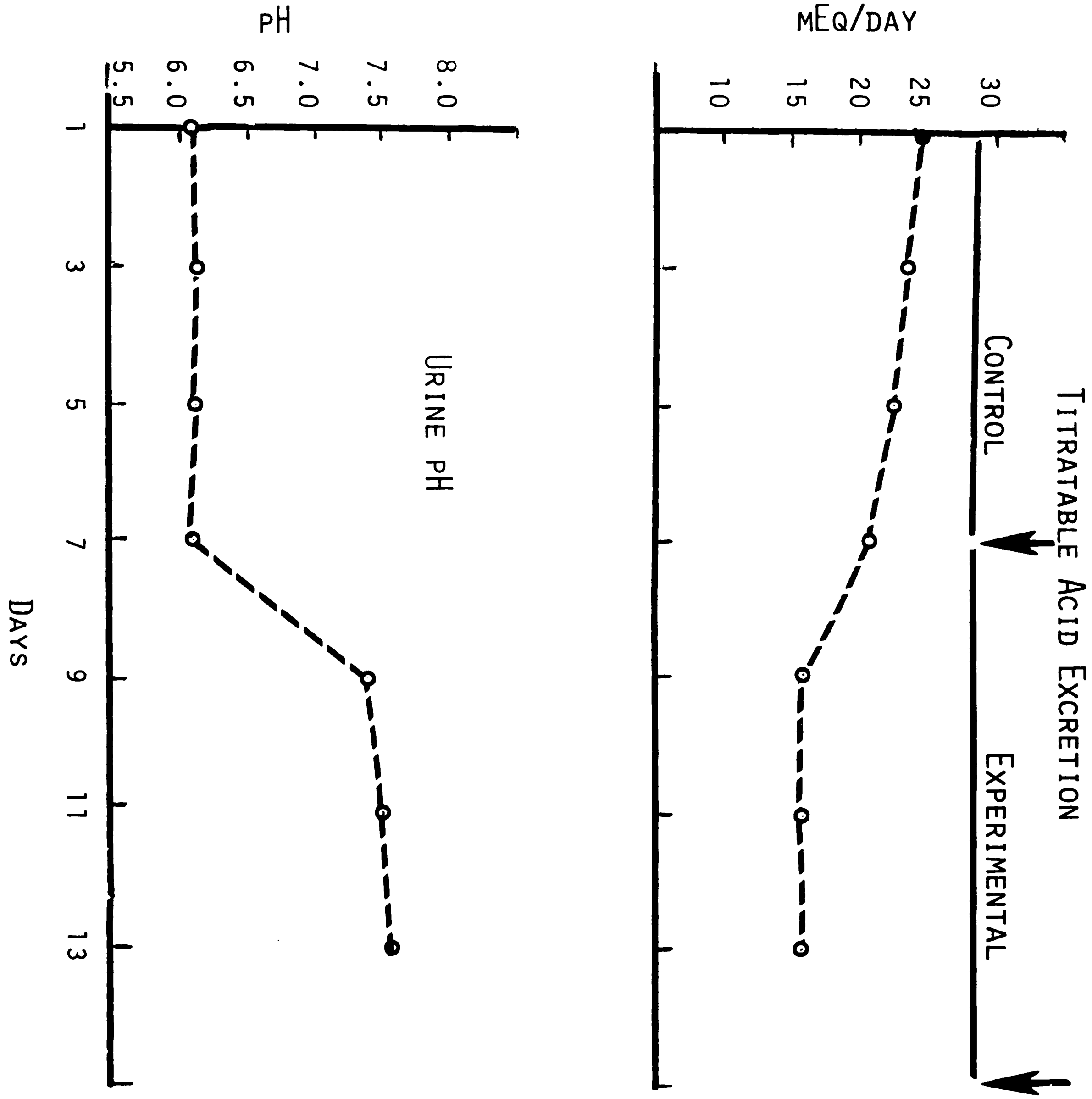
Although Lyon *et al.* stated that sodium bicarbonate was useful in treating patients with renal failure by compensating for the metabolic acidosis of renal failure, the studies in this thesis were performed on normal individuals and do not necessarily prove that sodium bicarbonate treatment would be useful for lowering SUN in uremics. Indeed the addition of sodium would be detrimental to those uremics who have difficulty in excreting sodium.

The overall concept that acid-base alteration can affect the SUN is substantiated by the experiments described in this chapter. Plasma creatinine showed no change during the ingestion of sodium bicarbonate. This observation seems to suggest that the GFR increase which occurs with the ingestion of sodium bicarbonate was not large enough to lower the serum creatinine concentration. No changes were observed in the levels of serum glucose and cholesterol. This suggests but by no means proves that sodium bicarbonate ingestion has apparently no effect on the metabolism of these two parameters of the serum.

The urinary pH (Table 4) was raised by the ingestion of sodium bicarbonate, while titratable acid was lowered.

In conclusion, the administration or ingestion of sodium bicarbonate has an effect on the lowering of SUN concentration in normal individuals. According to the observations made in this study, it is more likely that the effect may be either upon the urea production and/or the urea clearance.

Figure 2.--Average titratable acid excretion and urine pH of individuals fed typical American diet alone and with 15 g sodium bicarbonate.


Titratable Acid Excretion
Control
Experimental
mEq/day
30
25
20
15
10
Urine pH
pH
8.0
7.5
7.0
6.5
6.0
5.5
1
3
5
7
9
11
13
Days

Chapter II

Effect Of Ingestion Of Sodium Bicarbonate On SUN Of Individuals Consuming A Liquid Wheat Gluten Diet

Introduction

The purpose of this experiment was to repeat the studies performed by Taylor, *et al*. (114), paying special attention to the changes produced in the acid-base balance of the human subjects consuming the liquid wheat gluten diet. That group reported that there is an inverse relationship between the biological value of a protein and the level of blood urea. Hence, when wheat gluten was the only source of nitrogen, the blood urea increased in the human subjects. From the composition of the liquid gluten diet used in Taylor's study, it appears that it would produce an acidosis in the body - especially since cola drinks were consumed. If previous work of Lyon *et al*. (17) is valid, then possibly the acidic condition produced by Taylor's diet would counteract the reduction in urea levels that we have so frequently observed in subjects fed diets in which the only source of protein was from wheat.

This work involved a study to observe if the ingestion of sodium bicarbonate would lower SUN of normal individuals consuming the Taylor's liquid wheat gluten diet. This study would therefore determine (1) if acid-base changes were a possible cause of the alterations in SUN of individuals consuming wheat gluten as their source of dietary protein and (2) whether or not the SUN changes with changes in the acid-base state of individuals on an entirely different dietary regimen from the study with typical American diets described in the previous chapter.

Experimental Procedure

a. Control Period: Four normal, healthy, adult male subjects were fed a constant volume of the fluid diet described by Taylor's associates; Young _et al_. (181,182), Scrimshaw _et al_. (183). This was consumed in four equal doses at 8:00, 12:00, 17:00, and 21:00. This diet provided 2110 kilocalories. . .the remaining kilo calories needed to maintain body weight came from crackers containing flour, shortening and sucrose. Since Taylor _et al_. (114) indicated that soft drinks (mostly colas) were permitted _ad libitum_ with no definite indication of amount and brand, the subjects in our study were provided with 16 oz. of a specific soft drink (sprite, uncola) every day. By that means, the acidity attributable to the soft drink was the same in all subjects. Water was permitted, but the volume consumed was kept constant throughout the study for each subject. Each subject had in the refrigerator, a bottle containing a measured amount of water. All ingredients of the diet except for the soft drink were obtained from the same sources mentioned by Young _et al_. (181). The composition of the liquid diet is given in Appendix A. Body weights were recorded every other day throughout the entire study period (Appendix E). The control period was 7 days.

b. Experimental Period: The procedures carried out in the control period were also performed during the experimental period. The only difference was that a total of 10 grams of $NaHCO_3$ in 3 doses per day was consumed by each individual with water. This phase was extended for 7 days.

c. Sample Collection:

1. Urine

Every day during the study, 24-hour urine samples were collected by each subject. They were provided half-gallon, wide mouth, plastic bottles, which contained 10 ml. of toluene as a preservative. The urines were analyzed immediately after they were brought to the laboratory for total volume, pH and titratable acidity, as described in Chapter I of Part II of this thesis.

2. Blood

Each subject, before breakfast, reported to the Olin Health Center on the first and last days of the control and experimental periods. All blood samples were withdrawn from anticubital veins. After allowing the blood to stand for 4-6 hours in a refrigerator, serum was then isolated.

Analytical Procedure

A. Urine

The urine samples were analyzed in a similar manner as described in Part II, Chapter I.

B. Serum

The serum was obtained from the whole blood as described above and was preserved in the freezer until analyzed.

Serum urea nitrogen for each subject was determined by the diacetyl monoxine method and creatinine by Folin's picric acid method (184). Serum sodium and potassium concentrations were analyzed by an atomic absorption spectrophotometer, Perkin-Elmer 303 Model.

Results

Serum Urea Nitrogen (SUN)

SUN values in three individuals seemed to drop slightly when $NaHCO_3$ was taken with the liquid formula diets, despite the maintenance of a constant protein intake. One of the individuals whose control urine pH was high had an increase in his serum urea nitrogen levels when he ingested sodium bicarbonate (Table 6). The serum urea nitrogen levels were not significantly different at the end of the experimental phase as compared to those at the end of the control phase.

Serum Creatinine

The average concentration in the four subjects was the same at the end of the experimental (1.53 $\pm$ 0.8 mg/dl) as it was at the end of the control period (1.53 $\pm$ 0.10 mg/dl).

Urinary pH and Titratable Acid

As postulated, the liquid diet even without cola consumption seemed to produce an acid condition in the body; because urinary pH was on the acidic side when this diet

TABLE 6 .--Serum urea nitrogen (SUN) and urinary pH values of four normal adults who were on (1) liquid formula diet, according to Young *et al*., Am. J. Clin. Nutr. 26, 967, 1973, as control diet and (2) same liquid formula diet + 10 grams of $NaHCO_3$ in three doses per day.

Subjects	CONTROL SUN mg/dl	CONTROL Creatinine mg/dl	CONTROL Urinary pH	EXPERIMENTAL SUN mg/dl	EXPERIMENTAL Creatinine mg/dl	EXPERIMENTAL Urinary pH
D.W.	12.4	1.40	5.33	9.9	1.51	7.41
F.B.S.	17.9	1.51	6.00	20.6	1.44	7.50
C.B.	15.1	1.59	5.90	12.3	1.62	7.15
M.A.	14.6	1.63	5.20	13.6	1.58	7.32
Average	15.0±2.3[xxx]	1.53±0.10[x]	5.60±.40	14.1±4.5	1.53±0.80	7.30±.14[xx]

[x]Standard deviations are given for each average value.

[xx]Urinary pH at the end of experimental period is significantly ($P<0.05$) higher.

[xxx]There is no statistically significant ($P<0.05$) difference between the SUN values of control and experimental phases.

TABLE 7.--Urinary volume and acidity of four normal adults who were on (1) liquid formula diet, according to Young *et al.*, Am. J. Clin. Nutr. 26:967, 1973, as control diet and (2) same liquid formula diet + 10 grams of $NaHCO_3$ in three doses per day. Each dietary period lasted for a week. Values shown are average of last two days of each period.

Subject	CONTROL 24 hour Urine Volume ml	CONTROL 24 hour Urinary Acid Excretion mEq/day	EXPERIMENTAL 24 hour Urine Volume ml	EXPERIMENTAL 24 hour Urinary Acid Excretion mEq/day
D.W.	1210	122.9	1515	27.9
F.B.S.	1225	52.1	1715	17.4
C.B.	1350	105.5	1215	22.5
M.A.	1220	100.3	1580	28.9
Average	1251±66[x]	95.2±30.3	1506[xx]±211	24.2[xxx]±5.3

[x]Standard deviations are given for all average values.

[xx]Urine volume at the end of experimental period are significantly ($P<0.05$) higher than at

[xxx]Titratable acid excretion per day at the end of experimental period is significantly ($p<0.05$) lower.

TABLE 8 .--Serum sodium and potassium values of four normal subjects who were fed (1) liquid formula diets, according to Young *et al*., Am. J. Clin. Nutr. 26, 967, 1973, as control diet and (2) same *liquid* formula diet + 10 grams of $NaHCO_3$ in three doses. Each dietary period lasted 7 days. Blood samples were drawn at the end of each period.

	CONTROL		EXPERIMENTAL	
	Na^+ mEq/L	K^+ mEq/L	Na^+ mEq/L	K^+ mEq/L
D.W.	143	4.4	144	5.4
F.B.S.	144	4.7	144	5.9
C.B.	145	5.3	147	3.8
M.A.	144	4.7	144	7.0
Average	144±1.0^x	4.7±0.4	144±1	5.2±1.3

^{x}Standard deviations are given for all average values.

was consumed, the urine pH increased when $NaHCO_3$ was ingested with the same basic diet (Tables 6 and 7). Total titratable acidity of the urine was very high when the subjects were fed the control liquid formula diet and decreased significantly when a bicarbonate supplement was ingested with it. Urine volumes (Table 7) increased for 3 out of 4 subjects during the experimental phase. Titratable acidity which was 95.2 ± 30.3 mEq/day on the control liquid gluten diets dropped to 24.2 ± 5.3 mEq/day after ingestion of $NaHCO_3$ (Table 7) and (Fig. 3).

Serum Na and K

The serum sodium concentration was the same at the end of the control period as at the end of the experimental period despite the intake of an extra 119 mEq of sodium per day for 7 days. There was a slight increase in serum potassium levels after the ingestion of $NaHCO_3$ (Table 8). This was related to the fact that one serum sample was high in potassium because of hemolysis (M.A.).

Discussion

Three of the four subjects in this experiment showed a possible nonsignificant reduction in serum urea nitrogen when they ingested sodium bicarbonate (Table 6). Other researchers in the field of nutrition and medicine have reported that the administration of $NaHCO_3$ resulted in the reduction of SUN (177). The most probable cause of the discrepancy between the studies in this chapter showing no significant changes in SUN with sodium bicarbonate ingestion and the studies of others (176), and those in the previous chapter of this thesis, is that these subjects have an entirely different dietary regimen. Thus individuals on the typical American diet will have decreasing SUN when ingesting sodium bicarbonate and individuals on a nonmeat, wheat gluten source of dietary protein will have an insignificant change in SUN when sodium bicarbonate is ingested. No explanation is evident for this observation.

In this experiment, increased urine flow occurred as shown by the increased urine volumes in Tables 7 and 4. It can be suggested that the increased urine volume is caused by an increase in the glomerular filtration rate, which would also cause an increase in urea clearance. It is possible therefore that the decreased SUN at the end of the experimental period of the first experiment is caused by the increased urine flow and perhaps also GFR.

Furthermore, the previous investigators, on the basis of their observations, concluded that there was a decreased urea production when patients were given $NaHCO_3$ (177). The uremic acidosis is due to the retention of metabolically produced hydrogen ions. Accordingly, uremic acidosis is most widely viewed as the result of impaired excretion of titratable acid and ammonia. These ammonia and titratable acid excretion mechanisms are postulated to be responsible for renal conservation of fixed cation, i.e. sodium, and for the elimination of the proton of the acid produced during metabolism (136). That the plasma urea nitrogen is directly proportional to the body's acidity has been reported by various investigators (147,148). Whang and Reyes (176) also observed a significant decrease in the SUN of male Sprague-Dawley rats receiving $NaHCO_3$.

The precise mechanism responsible for the development of acidosis accompanying renal failure has not yet been completely established. One of the problems has been the application of a combination of dietary and other therapeutic measures to the patients which precludes the evaluation of any one variable. In the present two studies, none of the subjects were given any medication and there was no other dietary variable except the ingestion of $NaHCO_3$ during the experimental period.

In the present studies, all the subjects showed at least a small decrease in serum urea nitrogen with the exception of one individual (F.B.S.) in the second study. The increased SUN in this one individual may be due to the advanced age of the subject, who was 67 years old, while the rest of the subjects were 30 or younger. Researchers have reported a gradual diminution of the urea clearance and progressively increased blood urea nitrogen with advancing age. Also it should be noted that the control urine pH of this individual was noticably higher than that of the others on the same diet. Thus some unknown factor perhaps renal disease i.e. renal tubular acidosis was present in the individual.

The results of the present study agree with those reported by many investigators (177) as far as the effect of sodium bicarbonate on SUN is concerned. These previous reports indicate the administration of sodium bicarbonate even to normal subjects, whose SUN was well within the normal limits, causes a drop in the SUN concentration. The application of this phenomenon to the treatment of renal disease has been discussed by others (17).

Plasma creatinine concentration showed no change during treatment with sodium bicarbonate. There were some small fluctuations in specific individuals, but, on the average, there was no change before and after the treatments. This observation seems to indicate that the GFR increase which occurs with increased sodium bicarbonate ingestion was not large enough to lower the serum creatinine levels in these individuals.

In this study with the wheat gluten liquid diet, the serum samples showed no significant change in the concentration of sodium and potassium, resulting from the ingestion of $NaHCO_3$.

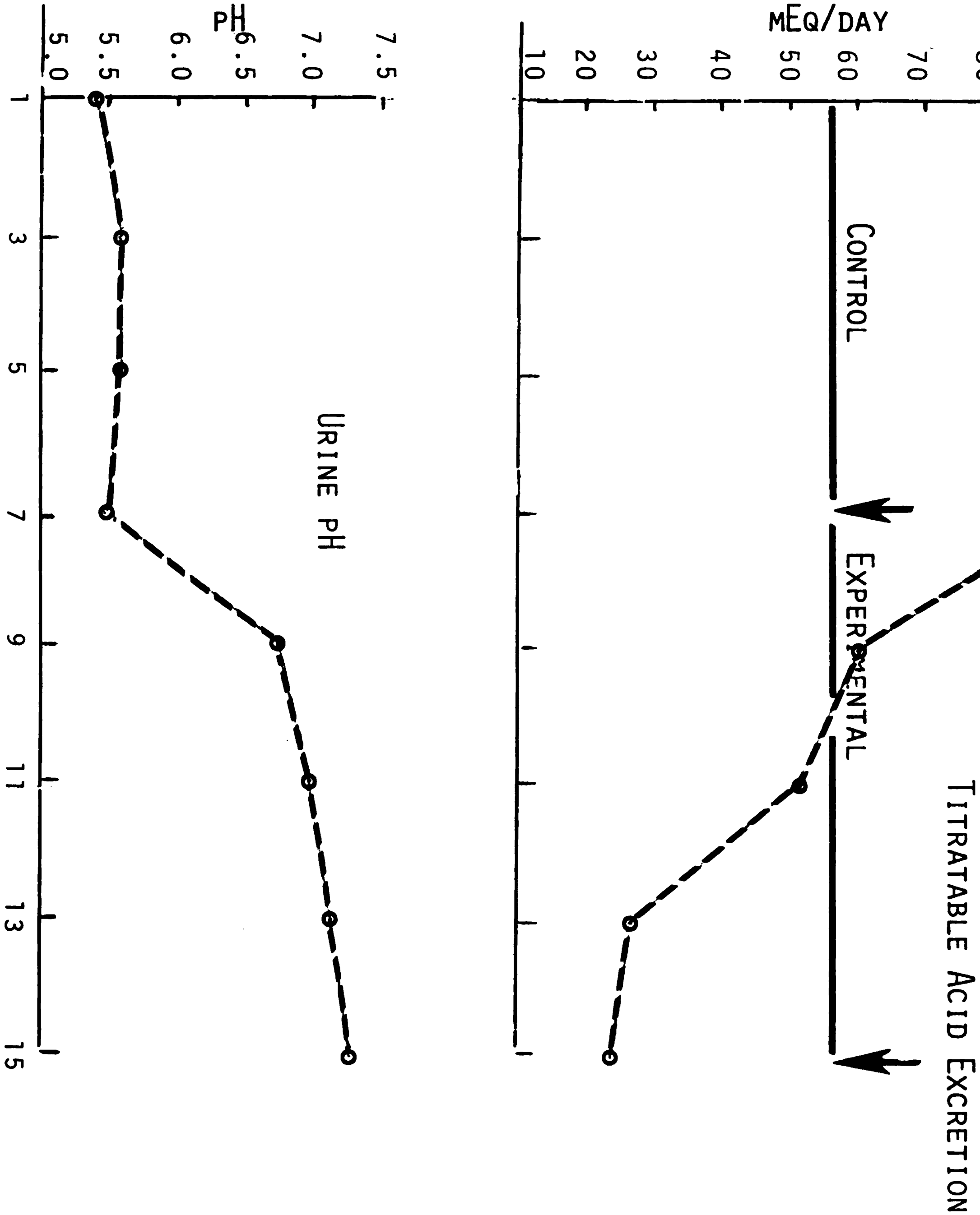
The body weights of these individuals recorded before the start of the study, at the end of control period and at the end of experimental period did not change and remained practically constant.

There was a slight increase in serum potassium concentration, as shown in Table 8, at the end of the experimental period. In one of the subjects, M.A., the increase in potassium concentration seems unusually high. The reason for this is probably that the blood was slightly hemolyzed, and part of the intracellular potassium may have been added to the serum. Normally, when individuals ingest $NaHCO_3$ their plasma potassium decreases because of alkalosis.

The urinary pH (Table 6) was raised by the ingestion of $NaHCO_3$, while titratable acidity, (Table 7) was lowered. According to Oser (178), under normal conditions, urine titratable acid excretion averages 35.0 mEq/day on a typical American diet. However, when the Taylor liquid formula was consumed by our subjects the titratable acidity was as high as 95.2 mEq/day. This confirms our assumption that metabolism of the Taylor liquid gluten diet produces above normal quantities of fixed acid. This observation therefore indicates that an alternative explanation for the increase in SUN in Taylor's original studies (114) may be that dietary production of fixed acid either decreases urea clearance or increases the urea production.

It is, therefore, concluded that the ingestion of sodium bicarbonate did not lower the SUN significantly in individuals consuming wheat gluten liquid diet. The possible explanation can be made that althouth the wheat gluten liquid produced a highly acidic urine, but did not produce the same conditions in the body as produced by a typical omnivorous American diet.

Figure 3.--Average titratable acid excretion and urine pH of individuals consuming Taylor's wheat gluten liquid diet, and the same diet plus 10 g sodium bicarbonate.



Titratable Acid Excretion
mEq/day
100
90
80
70
60
50
40
30
20
10
Control
Experimental
Urine pH
pH
7.5
7.0
6.5
6.0
5.5
5.0
1
3
5
7
9
11
13
15
Days

PART III

EFFECT OF THE NATURE OF DIETARY PROTEIN FED ISONITROGENOUSLY ON THE ACID-BASE HOMEOSTASIS AND ON VARIOUS PARAMETERS OF BLOOD AND URINE

Chapter I

The Comparative Effects Of Isonitrogenous And Isocaloric Diets On The Acid-Base Balance And Various Parameters Of The Blood And Urine Of Normal Human Subjects

Introduction

There is sufficient evidence both from our preliminary work and from reports in the literature to suggest that the acid-base condition in the body has a marked influence on a number of physiological and biochemical reactions. It should be emphasized that ingestion of meat because of its relatively high concentration of sulfur and phosphorus, tends to produce an acidic condition in the body; whereas vegetarian diets usually produce a more alkaline condition. These acid-base changes produced by different diets are reflected by changes in urinary pH and titratable acidity. The acid or base forming properties of food depend on the inorganic constituents or ash residue remaining after the food has been metabolized in the body; and, according to the type of the food eaten, the ash is neutral, acid, or alkaline.

On the basis of the preceeding studies, the experiments in this chapter were designed to explore (1) whether change from meat to potato diet alters serum urea nitrogen, creatinine and uric acid in particular, and other blood parameters which are determined by SMAC (186) automatically; and (2) whether change from meat to potato diet alters the urinary pH and titratable acidity. This study was initiated because it was suspected that shifting from meat to potato diet would make more alkaline metabolic products. By making the body more alkaline, it was suspected that serum urea nitrogen would decrease on the potato diet. If this assumption were to be validated by these experiments, this finding

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would substantiate the proposal that acid-base changes in the body affect the SUN and also would suggest that the decreased BUN observed in individuals on wheat diet (5) may also be caused by alkaline dietary metabolic products.

Experimental Procedure

To determine that a vegetable source of dietary protein can change the SUN, two experiments were performed as follows. The protocol and diets were the same; in that they were isonitrogenous and isocaloric. The major source of protein during the control period, in both cases, was meat and during the experimental period, it was potato. The purpose of the first experiment was to explore whether a vegetarian diet has an effect on blood urea nitrogen and acid-base balance as reflected by the urinary pH. In this first study with three subjects, only a few blood parameters were examined; and only the pH of the urine was determined. In the follow-up experiment, about six months after the first, six college students were chosen for the study. Numerous serum parameters were measured. Also in this second study, urinary titratable acid excretion and urine flow rate were measured along with the urine pH.

Subjects

In all, there were ten volunteers who participated in these two experiments. One of the subjects in the first experiment caught flu and was dropped. The rest of the subjects remained healthy and had no complaints throughout the entire study. The subjects had no significant change in body weight (Appendix F).

Schedule

Both studies consisted of a control period of seven days and an experimental period of seven days. Twenty-four hour urines were collected every other day.

Blood samples were drawn at the beginning and end of the control period and then at the end of the experimental period.

All meals were provided to the volunteers at no cost.

Diets

During the control period, the subjects received a variety of foods which provided 46 g of protein per day per person regardless of the subject's size. The major source of the protein was meat. The amount of protein given during the control period was chosen so that it would be isonitrogenous with that in the experimental diet. The caloric intake in the control period was raised by providing extra carbohydrates (Table 9a), to make both the diets approximately isocaloric. The subjects were required to consume all of the food provided.

The diet of the experimental period also contained 46 g of protein per day per person. However, the major source of protein on this experimental diet was potatoes. The subjects were required to consume all the food provided in this experimental diet including the drippings of mutton tallow, which were a major source of calories.

TABLE 9a.--Composition of meat diet (control)

Menu: This was based on Mrs. Mickelsen's Formula II dated Feb. 7, 1975. The object of the menu was to provide 123 g of lamb fat or 1107 kcal of fat in the diet containing 2800 kcal total. Each person received Formula II in the following amount:

	Kcal	Protein (g)
200 g lean meat containing: 17% protein	120	34
5% fat	90	-
114 g mutton fat	1026	-
Ordinary bread (with calcium) 6 slices	420	12
Jam, maple syrup, honey, hard candy	300	-
Dripping, mutton tallow (on bread) 15 g	135	-
2 oranges or apples	80	-
lettuce or other greens	40	-
	2211	46
2 cups coffee or tea per day (no sugar or cream)		
Honey (1st exp., 3 subjects) 2 tablespoons per day		
Honey (2nd exp., 6 subjects) 1 tablespoon per day		
Sprite 12 oz per day		
Carbohydrates added specifically to increase caloric intake of meat diet		
1st exp weighed amounts of candy and sugar		
2nd exp weighed amounts of chocolate fudge	617	
Total	2828 Kcal	

TABLE 9b.--Composition of potato diet (experimental)

	Kcal	Protein (g)
1032 g potato (boiled and peeled)	785	21.7
123 g mutton fat	1107	-
127 g potato concentrate	446	10.6
7 slices bread	490	14.0
Total	2828	46.2

2 oranges or apples, honey, Sprite, lettuce, coffee or tea was consumed in same amount as given during the control diet.

Both diets were cooked in the laboratory by a volunteer. The complete dietary intakes for the control and experimental periods are shown in Tables 9a and 9b. The same daily menu was followed throughout the entire study.

Sample Collection

Twenty-four hour urines were collected by each individual every other day. They were provided with wide mouth, plastic bottles containing toluene, as a preservative. A plastic bag was provided so that the subjects could carry their bottles.

Blood samples were drawn in the Olin Health Center and after four-six hours standing in a refrigerator; they were centrifuged for serum collection. All blood samples were collected from the anti-cubital vein before breakfast. Serum samples were preserved by freezing.

Analytical Procedure

A. Urine

As the urine samples were brought to the laboratory, they were examined for the pH and titratable acidity as described previously on page 60.

B. Serum

The frozen serum samples were sent to the Laboratory of Clinical Medicine, where they were analyzed by SMAC (186) for SUN, creatinine, uric acid, Na^{+}, K^{+}, Ca^{++}, P^{+++}, total protein, total CO_2, glucose, cholesterol, and albumin. Since the serum was exposed to the atmosphere, the values for total

CO_2 are not valid. Also since the blood pH was allowed to change after it had been collected, serum potassium level may also not be valid. Since no metabolic inhibitor was added to the blood to prevent glucose metabolism, the serum glucose analysis is probably also not valid.

C. Diet

The protein and caloric contents of the diet were calculated from the U.S. Agriculture Handbook, No. 8.

Results

Effect of Potato Diet on Serum Urea Nitrogen

The average SUN changed slightly from 14.2 mg/dl at the start of the control diet to 13.3 ± 4.7 mg/dl at the end of the control period. The change is not statistically significant. But, when the subjects consumed the potato diet which was isonitrogenous and isocaloric to the control diets, the serum urea level in each subject decreased (Table 10). The decrease was about 50% on an average. At the end of the seven day experimental period, the average SUN values were 7.0 ± 1.3 mg/dl as compared to 13.3 mg/dl at the end of the control (meat) diet.

In the follow-up experiment with six college students, when the approximately same dietary regimen was followed, the SUN values (13.0 ± 4.0 mg/dl) at the beginning of control increased slightly toward the end of the control diet to 14.8 ± 3.0 mg/dl. There is nothing to suggest that this slight change was of any physiological significance or that it is statistically significant. At the end of the experimental period, the SUN was observed to be lower than the respective control SUN in each of the six individuals. The reduction amounted to about a 30% decrease in average values for the serum urea nitrogen. The average value dropped from 14.8 ± 3.0 mg/dl at the end of the control diet to 10.3 ± 1.6 mg/dl of serum at the end of seven days on the potato diet (Table 11).

Serum Creatinine and Uric Acid

In the second experiment (Table 11), there was no change in the concentration of creatinine between the end of the control phase, 1.0 ± 0.1 mg/dl and the end of the experimental phase, 1.0 ± 0.1 mg/dl. On the other hand, uric acid concentration may have slightly decreased on the potato diet as compared to the meat diet; however, the observed change is not statistically significant. Serum uric acid which was 6.9 ± 1.4 mg/dl at the end of the meat diet decreased to 5.7 ± 0.8 mg/dl at the end of the potato diet.

Urinary pH and Titratable Acidity

In both experiments, the urinary pH changed significantly when the experimental diet was consumed by the subjects (Tables 10 and 12). The urinary pH in the first experiment in all three subjects changed from acid to alkaline. At the end of the control phase, the urinary pH was 5.5 ± 0.3 and at the end of the experimental phase, it increased to 7.3 ± 0.1 (Table 10). In the second study, the pH of the urine which was 5.8 ± 0.2 at the end of the

TABLE 10.--Serum values for urea nitrogen and urinary pH of normal adult males. Three subjects were fed iso-nitrogenous diets (1) mutton (control) for 7 days, followed by (2) potato (experimental) for 7 days.

	CONTROL				EXPERIMENTAL			
	Trigly-ceride mg/dl	Choles-terol mg/dl	Urea Nitrogen mg/dl	Urinary pH	Trigly-ceride mg/dl	Choles-terol mg/dl	Urea Nitrogen mg/dl	Urinary pH
D.W.	136	234	8.5	5.30	192	213	6.0	7.44
M.A.	100	267	12.5	5.83	92	222	6.5	7.22
F.B.S.	100	291	18.0	5.87	92	213	8.5	7.31
Average	112±20	264±28	13.3±4.7	5.50±0.30	125±57	216±5	7.0±1.3	7.30±0.10

SUN is significantly higher and urine pH is significantly lower on control diet than the experimental diet ($P<0.05$).

*Standard deviations are given for all average values.

TABLE 11. Effects of isonitrogenous and isocaloric diets based on (1) meat and (2) potato

TABLE 11.--Effects of isonitrogenous and isocaloric diets based on (1) meat and (2) potato diets, respectively on serum levels of urea and creatinine and carbon dioxide and uric acid of human subjects. Each dietary period lasted for 7 days.

Subjects	MEAT DIET				POTATO DIET			
	CO_2^{Δ} mM/L	Urea-N mg/dl	Uric Acid mg/dl	Creat-inine mg/dl	CO_2^{Δ} mM/L	Urea-N mg/dl	Uric Acid mg/dl	Creat-inine mg/dl
J.I.	20	16.0	6.0	1.3	25	10.0	4.4	1.2
D.K.	25	16.0	8.1	1.1	26	13.0	6.3	1.0
D.L.	24	16.0	7.5	1.3	25	11.0	5.5	1.3
T.O.	22	14.0	5.4	1.0	25	10.0	5.9	1.0
S.W.	21	14.0	5.5	0.9	20	10.0	5.4	0.9
D.W.	22	10.0	8.9	0.9	24	8.0	6.9	0.9
Average	22±1*	14.8±3.0	6.9±1.4	1.0±0.1	24±2	10.3±1.6	5.7±0.8	1.0±0.1

SUN values are significantly lower on the potato diet ($P<0.05$). Potato diet serum CO_2, uric acid, and creatinine were not statistically significant from meat diet serum values.

*Standard deviations are given for all average values.

ΔThe total CO_2 values on this table are not valid.

TABLE 12.--Effect on urinary acidity of individuals fed isonitrogenous and isocaloric diets based respectively on (1) meat and (2) potatoes. Each dietary period lasted 7 days.

Subject	MEAT DIET		POTATO + BREAD DIETS	
	Urinary pH	Titratable Acid Excretion mEq/day	Urinary pH	Titratable Acid Excretion mEq/day
J.I.	6.0	35.5	7.0	10.3
D.K.	5.5	19.0	7.0	12.0
D.L.	5.7	16.8	8.2	9.3
T.O.	6.1	17.7	7.4	13.5
S.W.	5.7	15.3	7.4	8.7
D.W.	6.0	20.1	6.6	10.0
Average	5.8±0.2	20.7±7.4$^{\Delta}$	7.2±0.5	10.6±1.8$^{\Delta}$

*Standard deviations are given for all average values.

ΔStatistically significant from meat diet values ($P<0.05$).

TABLE 13.--Effect on urinary volume of individuals fed isonitrogenous and isocaloric diets based respectively on meat and potato. Values shown are the averages of the 3 day urine collection of each dietary period.

Subjects	ml/day				
	MEAT DIET			POTATO + BREAD DIET	
	Start	Middle	End	Middle	End
J.I.	750	820	680	1060	940
D.K.	690	780	400	700	610
D.L.	520	645	575	1100	1025
T.O.	610	900	440	550	630
S.W.	350	620	325	660	525
D.W.	1400	1010	1100	1095	1300
Average	720 ± 361^{x}	795 ± 149	$586^{**} \pm 281$	860 ± 250	$838^{**} \pm 300$

*Standard deviations are given for all average values.

**Urine volume on potato diet are significantly higher as compared to meat diet ($P<0.05$).

TABLE 14.--Effect on serum electrolytes of individuals fed isonitrogenous and isocaloric diets based on (1) meat and (2) potato diets. Each dietary period lasted for 7 days in each case.

	MEAT DIET					POTATO + BREAD DIET				
Subjects	Cl- mEq/L	Na+ mEq/L	K+ mEq/L	Ca++ mg/dl	P+++ mg/dl	Cl- mEq/L	Na+ mEq/L	K+ mEq/L	Ca++ mg/dl	P+++ mg/dl
J.I.	109	146	4.5	9.6	3.5	104	143	4.0	9.6	3.8
D.K.	103	143	4.2	10.0	2.9	105	144	4.2	10.0	3.6
D.L.	102	140	4.4	10.1	3.2	102	141	4.8	10.2	3.5
T.O.	106	144	4.7	10.6	4.1	106	143	4.7	10.3	4.2
S.W.	105	143	4.0	10.2	3.4	105	141	4.4	9.9	3.4
D.W.	105	142	4.5	10.1	3.5	106	146	4.6	10.4	4.0
Average	105 ±2*	143 ±2	4.3 ±0.2	10.1 ±0.3	3.4 ±.3	104 ±1	143 ±1	4.4 ±0.3	10.0 ±0.3	3.7 ±0.3

*Standard deviations are given all average values.

TABLE 15.--Effect on serum levels of proteins of human subjects fed isonitrogenous and isocaloric diets based respectively on (1) meat and (2) potato diets. Each dietary period lasted 7 days.

Subjects	MEAT DIET		POTATO + BREAD DIET	
	Total Protein g/dl	Albumin g/dl	Total Protien g/dl	Albumin g/dl
J.I.	6.9	4.2	6.9	4.2
D.K.	7.0	4.5	7.0	4.7
D.L.	7.0	4.5	6.9	4.5
T.O.	7.7	5.0	6.8	4.7
S.W.	7.7	4.8	7.4	4.6
D.W.	7.3	4.9	7.2	4.8
Average	7.2±0.3*	4.6±0.3	7.0±0.2	4.5±0.2

*Standard deviations are given for all average values.

TABLE 16.--Effects of isonitrogenous diets based respectively on (1) meat and (2) potato + bread on blood and urine composition of human subjects.

Subjects	MEAT DIET (7 days)		POTATO + BREAD DIET (7 days)	
	Serum Triglyceride mg/dl	Serum Cholesterol mg/dl	Serum Triglyceride mg/dl	Serum Cholesterol mg/dl
J.I.	190	178	90	157
K.D.	76	192	43	182
D.L.	152	195	334	203
T.O.	104	281	138	288
S.W.	100	221	304	201
D.W.	118	245	148	236
Average	106±26	218±38	176±117	221±45

control period rose to 7.2 ± 0.5 at the end of the experimental period (Table 12). This shows the alkalinizing effect of the potato diet. Similarly, total titratable acidity of the 24 hour urine which was 20.7 ± 7.4 mEq/day during control dropped to 10.6 ± 1.7 mEq/day as the urine became more alkaline at the end of the experimental potato diet. The reduction in acidity was accompanied by a slight increase in the average daily urinary volume (Table 13). The time course of the change in urine pH and titratable excretion is shown in Figure 4.

Electrolytes and Protein

There was no change observed in the serum electrolyte concentration throughout the entire study (Table 14). Furthermore, the total serum protein and serum albumin remained constant (Table 15).

Discussion

In two different experiments with human subjects, diets high in potatoes and free from animal protein produced a reduction in serum urea levels and an increase in urinary pH. This was true when the subjects were fed diets, in which the sole source of protein was potatoes and bread as compared to an isonitrogenous, typical American, omnivorous diet. The influence of different sources of dietary protein on the blood urea level was reported in 1931 by Lyon in Edinburgh (18). They clearly demonstrated the reduction in blood urea nitrogen when their patients were shifted to diets wherein the protein was derived from vegetables. These authors interpreted the decreased BUN as being caused by change in the protein from animal products to vegetable products. They also suggested that body acid-base changes with different diets may also influence the BUN. The results of the experiments described in this chapter support the latter (acid-base) proposal of Lyon.

In the study (Table 10) with three subjects, there is a reduction of almost 50% in the serum urea nitrogen after 7 days on the isocaloric, isonitrogenous potato diet. At the same time, urinary pH on potato diet increased to the point where the urine became alkaline. The potato diet was free from meat which is rich in sulfur and phosphorus. Thus, the potato diet produced alkalinity in the body, as reflected by increased urine pH.

In a follow-up study, with six normal, young college students, the dietary regimens, shown in Table 9a and 9b, were followed strictly. In this second study, a similar reduction (30%) in serum urea levels was observed when the experimental diet was fed (Table 11). This SUN reduction was, again, accompanied by increased urinary pH when the experimental potato diet was fed. These observations further substantiate results of other investigators, who have presented evidence that alkaline diets reduce SUN (18). In the studies described in this thesis, the cause of the decrease in urea nitrogen seems to be extra-renal rather than due to changes in the renal function because serum creatinine and uric acid were not altered. It should be emphasized however, that changes in renal function can take place without significantly altering serum creatinine and uric acid concentrations.

When the diet was changed from meat to potato, there seemed to be an effect on the rate of urine flow. The 24 hour urine volume was 40% higher on potato diet as compared to meat diet (Table 13). From the increase in urine flow on potato diet, it can be suggested that the reduction of SUN on potato diet might, in the past, be due to an increased urea clearance, brought about by increased flow rate in the nephron.

Previous reports from this laboratory have indicated that there is no change in the fecal nitrogen, or urinary urea nitrogen, when the subjects were changed from a typical American diet to diets high in wheat (5). Thus, the change brought about by the nature of dietary sources of protein seems to have no influence upon the production rate of urea. On the other hand, others have observed a decrease in liver arginase activity when rats ingest protein from wheat as compared to milk protein, casein (113). This latter observation suggests that urea production in the rat is lower when wheat is the source of dietary protein. Thus, there is conflicting evidence concerning the rate of urea production by the liver when animals have different dietary sources of protein. Since the studies performed in this thesis had no urea analysis performed on the urine, no further information regarding rate of urea production by the body can be given.

Although there seemed to be a decrease in the concentration of uric acid in the serum when the subjects consumed potato diets (Table 11), this change was not statistically significant. It is possible that, due to the impaired uric acid excretion in acidosis (186-191), there was less uric acid excretion on the control diet.

No changes in serum electrolyte concentration occurred between the end of the control and the end of the experimental periods (Table 14). These observations indicate that calcium, phosphorous, Na and Cl control was maintained during the dietary changes. The lack of change in the serum potassium concentration, suggests that only small acid-base changes occurred as a result of the shift in meat to potato diet. Acidosis usually increases and alkalosis decreases serum potassium; however, in this study, the subjects consuming the potato diet had approximately seven times the potassium intake of those on the meat regimen. Therefore, it is possible that the high potassium intake of the subjects on the potato diet raised their serum potassium levels in spite of their alkalotic condition. The obviously higher intracellular potassium levels of the individuals consuming the potato diet would probably have a profound effect on their body chemistry. Whether it can alter SUN is unknown.

Total protein and albumin levels in the subjects' blood on the two different diets remained constant. Since changes in serum protein levels usually occur rather slowly, the constancy of the total protein and albumin levels during the course of the study is not surprising. This observation also suggests that the proteins from the potato used in this study were of such biologic value that no drastic changes in protein synthesis resulted.

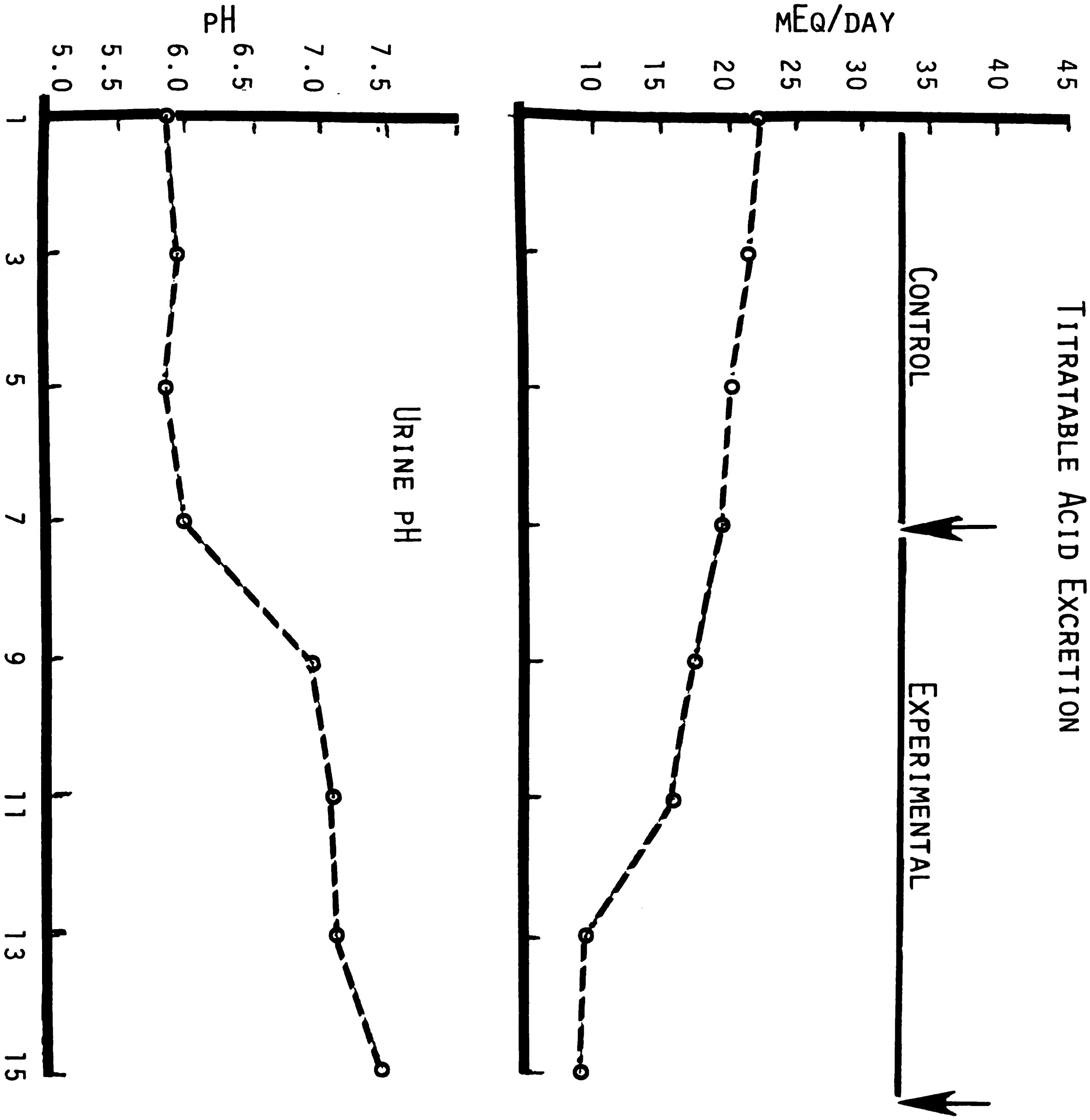
Although the only statistically significant changes observed in this study were decreases in SUN, decreased urine titratable acid excretion, and perhaps increased urine flow rate, it is possible that an infinite number of other changes also took place. When the body becomes more alkaline: intracellular potassium increases, plasma bicarbonate increases, renal bicarbonate excretion increases, ionic calcium decreases, urinary ammonia excretion decreases, hepatic glutamine synthesis decreases, etc. Therefore, the possibility exists that any one of an infinite number of alterations in the body can be the cause of the decreased SUN.

Thus, it appears that the reduction in serum urea level associated with the potato diet may be due to either a decrease in its production or increased clearance. Although there is still confusion as to the effect of the nature of dietary protein on SUN, there appears to be agreement that a vegatarian diet which produces an alkaline-type reaction in the body, if consumed for some time, results in a reduction in serum urea levels (18).

Such a relationship was suggested by several investigators (18). Although the change observed in this study seems to show a relationship between the alkalinization of the body as reflected by a decreased titratable acidity of the urine and reduction in serum urea levels, the physiological and biochemical significance thereof is still unknown.

In conclusion, the data obtained as a result of these studies suggest that the changes of acid-base balance in human subjects has an effect on SUN concentration. If the alkalinizing diets of plant origin are consumed, they lower the serum urea concentration significantly.

Figure 4.--Average titratable acid excretion and urine pH of 6 human subjects who were fed isonitrogenous and isocaloric (1) meat and (2) potato diets respectively.


Titratable Acid Excretion
mEq/day
45
40
35
30
25
20
15
10
Control
Experimental
Urine pH
pH
7.5
7.0
6.5
6.0
5.5
5.0
1
3
5
7
9
11
13
15

Chapter II

Serum Urea Levels In Adult Rats Fed Isonitrogenous Rations Which Provided 10% Protein From Casein Or Wheat

Introduction

Urea metabolism under normal conditions appears susceptible to a variety of factors other than changes in dietary protein level. The production and excretion of protein metabolites reflects the sum of many metabolic processes in the body. Nitrogen end-products, of which urea is the major constituent, added to the blood and excreted through the kidneys are, to a large extent, the result of an interaction between the diet and the organism.

Normally, the blood urea level in healthy individuals is controlled by several factors. So far, the quantity of protein has been the main aspect of this problem that has received any attention by investigators interested in nutrition. However, there are very few instances in which the correlation between urea level and the quality of dietary protein was intentionally studied separately from dietary protein level.

The present experiment was undertaken to examine whether or not there is an effect on the serum urea levels when two different kinds of protein are fed at the same level of dietary nitrogen. For that, healthy, adult male rats were fed isonitrogenous rations which contained 10% protein either from casein or wheat.

Experimental Procedure

Animals

Adult, Sprague-Dawley male rats were used for this study. The rats weighed between 250 and 300 g. The reason for selecting adult rats was that earlier workers using weanling rats fed different kinds of protein secured results which were not directly applicable to human subjects. The nutrient requirements of growing rats are entirely different from those of the adult, especially in quantitative terms. The rats were divided into two groups with six rats in each group. In order to give them an adjustment period, these rats were put on two different diets for four weeks to establish the same sort of nutriture prior to the actual experiment.

One of these was the grain ration which contained 23% protein and the other was the casein ration which had 20% protein. At the end of the four weeks, those rats fed the grain ration were transfered to the whole wheat ration which had 10% protein (Table 18) while those fed the casein ration were given the casein ration which contained 10% protein (Table 18). Both of the 10% rations were fed for four weeks.

Rations

The composition of the diets are shown in Tables 17,18 The whole wheat grain was ground to medium fine flour. The other ingredients were added, in amounts shown in Table 18 The rations were prepared by thorough mixing in an electric mixer. Before the rats were caged separately, they were

TABLE 17.--Proximate composition of the casein and grain rations.

	Casein Ration	Grain Ration[1]
	%	%
Protein	20% (from casein)	23.4
Fat	5% (corn oil)	3.0
Carbohydrates (added or by difference)	66.0 (cornstarch)	53.5
Fiber or cellulose	2.0	3.8
Moisture	-	10.0
Ash	5.0	6.3
Vitamin mixture	2.0	-
	100%	100%

[1]The ingredients in the grain ration are listed by Schemmel, et al., (192)

TABLE 18.--The composition of isonitrogenous casein and wheat rations (10% protein level) used in the rat studies.

	Casein Ration	Wheat Ration
	%	%
Casein[1]	11	-
Whole wheat (ground)[2]	-	80
Corn starch	75	11
Vitamin mixture[3]	2	2
Mineral mixture[4]	5	4
Corn oil	5	3
Cellulose[5]	2	-
	100%	100%

[1]Casein was purchased from General Biochemicals, Chagrin Falls, Ohio. The casein contained 91.25% protein.

[2]Wheat was obtained from the Department of Crop Science, Michigan State University (Genesee variety of wheat)

[3]Vitamin mixture purchasef from Nutritional Biochemicals Corporation, Cleveland, Ohio.

[4]Mineral mixture was prepared according to A.O.A.C. (193).

[5]Cellulose, microcrystaline, from Nutritional Biochemicals Corporation, Cleveland, Ohio.

weighed and paired on the basis of their weights. After four weeks, the rats getting the 20% casein rations were put on the ration containing 10% protein from casein and the other group fed the grain ration was then fed the ration containing 10% protein from wheat. The composition of these rations is shown in Tables 17 & 18. These rats were fed their proper rations, ad libitum, for four weeks; water was available all the time. At the end of the experiment, blood samples were secured by cardiac puncture under light ether anesthesia. The blood samples were drawn five hours after the feeding cups were removed from the cage, so that they were in a fasting state.

During the initial 4 week period, daily food consumption was recorded. After four weeks of ad libitum feeding, the rats were continued on the same protein rations but were pair-fed for an additional four weeks; water was available all the time. At the end of the period, blood samples were drawn.

Serum was obtained from the blood samples and the sera analyzed for urea nitrogen and creatinine concentration.

Analytical Procedure

A. Blood

The serum was preserved in a freezer until analyzed. The sera were analyzed for urea nitrogen concentration and creatinine as described in previous experiments.

Results

Weight Gain

While the rats were fed the 20% protein rations containing either casein or grain, they gained weight quite rapidly. After four weeks on the 20% protein rations, when the rats were fed isonitrogenous casein and wheat rations at a 10% protein level, they also gained weight, but at a slower rate. The rats either maintained or gained a little weight in both groups when they were pair-fed. The results are shown in Table 19.

Serum Urea Nitrogen

When the rats were fed ad libitum, the serum urea nitrogen (Table 20) was lower in the group consuming the wheat ration as compared to those fed the casein ration ($P<.05$). A similar difference in SUN levels occurred during the paired feeding period ($P<.05$).

There was essentially no change in the serum creatinine values both during ad libitum and paired feeding periods. That was in contrast to the urea levels which were significantly lower when the animals were fed the wheat ration despite the fact that both it and the casein ration had the same level of protein. The SUN concentrations of the rats fed the wheat ration were significantly lower than those fed the casein ration in both cases, i.e., ad lib and paired feeding. The SUN values of 10% casein fed group were 14.0 mg/dl and 14.2 mg/dl on ad libitum and pair feeding periods, while the rats fed the 10% wheat protein ration

TABLE 19.--Weight in grams of rats fed rations containing 10% protein from casein or wheat rations. The animals were fed ad libitum or pair fed for four weeks.

	Approx. 20% Protein level in ration				10% Protein level in ration			
	Ad lib fed				Ad lib fed		Pair fed	
	Initial		2 weeks on 20% Protein		3/24/76 to		4/19/76 to	
Dates	3/9/76		3/24/76		4/19/76		5/12/76	
Rats	Casein	Grain	Casein	Grain	Casein	Wheat	Casein	Wheat
1	310	319	370	410	400	410	405	425
2	345	353	407	422	425	453	432	460
3	357	368	434	465	454	481	459	471
4	350	325	447	381	492	440	495	440
5	350	345	419	428	455	460	463	437
6	353	355	435	453	468	455	475	460
Average weights (g)	344±17[x]	344±18	418±27	426±30	449±32	449±23	454±32	448±17

[x]Standard deviations are given for each average value.

TABLE 20.--Serum urea nitrogen (SUN) and creatinine in two groups of rats fed isonitrogenous casein or wheat rations (10% protein) both ad lib and pair-fed for a period of four weeks. All values are given in mg/dl.

	AD LIB				PAIR-FED			
	Casein Group		Wheat Group		Casein Group		Wheat Group	
	SUN	Creatinine	SUN	Creatinine	SUN	Creatinine	SUN	Creatinine
	13.8	1.05	11.8	1.07	13.8	0.98	11.0	1.07
	13.9	1.05	11.4	1.04	14.1	1.0	12.0	1.07
	14.1	1.03	11.6	1.06	13.6	1.01	11.5	1.03
Average	14±0.1[x]	1.04±.01	11.6±0.2	1.05±.01	14±.2	0.99±.01	11.5±.5	1.05±0.02

In both cases, ad lib and pair fed, the serum urea nitrogen was significantly lower in the wheat-fed than in the casein-fed animals ($P<.05$).

[x]Standard deviations are given for all average values.

had SUN values of 11.6 mg/dl and 11.5 mg/dl on paired feeding and ad libitum feeding respectively. The values for the rats in the latter two groups were all below the lowest value for the casein-fed animals.

Discussion

The consumption of a wheat flour ration by adult rats, weighing more than 300 g each, produced a lower SUN level as compared to that seen in rats fed an isonitrogenous casein ration. The reduction in serum urea nitrogen brought about by the wheat ration occurred both in the ad lib and pair-fed periods.

When the SUN was compared, the group fed the wheat ration both in the ad libitum and pair-fed periods had lower values than the casein fed group (Table 20).

Our results confirm those of Sterk _et_ _al_. (112). They reported the blood urea concentration of pigs fed the ration containing animal protein to be higher than that of the animals fed rations containing plant protein.

Although we observed a lower serum urea nitrogen value in the animals fed the wheat ration in comparison to the levels in the casein-fed rats, we did not see any change in the creatinine concentration of the serum.

In this study, both types of rations produced the same rate of weight gain. The present study shows that the reduction in SUN levels cannot be attributed to any difference in nitrogen intake from the casein or wheat rations.

The rations were consumed by the rats in equal quantities which was about 22 g per rat per day. The difference in SUN levels may be related to variations in digestibility of the protein or to alterations in urinary urea nitrogen excretion or to other factors. One explanation for the reduction in SUN levels involves a reduction in the production of urea which may be influenced by the urea cycle enzymes. Kiriyami et al. (113) reported that the urea excretion of the gluten fed group of rats was significantly higher than that of the casein fed groups, though nitrogen intakes were almost the same for both groups of the same age. Not only this, they also observed that the urea production and liver arginase activity of the casein fed group was significantly higher as compared to the gluten fed group. Nitsan and Liener (194) reported the levels of urea in the blood and urine of rats fed raw and heated soya bean meal, differing in protein quality. They could not find a difference in the level of urea in the blood and urine of the animals fed autoclaved or raw soya bean meal. They suggest that poor quality raw soya bean has no effect on the SUN.

Schimke (100) demonstrated the most striking differences in two groups of rats in their urea excretion when fed different diets. At the beginning of the experiment, animals on protein-free diets excreted 418 mg of urea per day; this amount was reduced to 102 mg per day on the fourth day of the experimental period, and to 87 mg per day on the seventh day. The urea excretion of the starved rats, in contrast, rose from 418 mg per day at the beginning of the experimental period to 962 mg per day for the fourth day of starvation and rose further to 2010 mg per day on the seventh day of the experiment. These observations indicate that the level of urea cycle enzymes in the liver is not related to the total protein, but is the function of the rate of protein breakdown.

It seems that urea metabolism appears susceptible to a variety of factors other than the changes in protein level of the diet, which may include fasting or caloric deficiency.

Eggum (108) reported that SUN is inversely proportional to the biological value of the protein in the ration. He tested about 42 different proteins, which included cereal, oil cakes, etc.; they were incorporated into rations which were fed to weanling rats. That investigator ignored the fact that the nutrient requirements are entirely different for both adult rats, weanling rats, and human infants (195). Moreover, the rats were fed ad lib, consequently food consumption varied tremendously from ration to ration. Thus, the nitrogen and the caloric intakes must have been variable. In addition, such observations were made during a short experimental period, which lasted for only 5 days in each case.

On the other hand, work by Nitsan _et_ _al_. (110) showed a reduction of blood urea nitrogen in adult rats fed a wheat ration which was isonitrogenous to a casein diet. The observations made in this study, confirm the results secured by Nitsan *et al*. (196). This reduction could not be explained since Nitsan *et al*. (110), reported only a slight or no change as far as urinary nitrogen or urea excretion was concerned.

Although the wheat ration produced a reduction in the SUN level in adult rats, Nitsan *et al*. (110) did not observe a comparable change in weanling rats fed the same rations. That probably explains the observations of Eggum (108) which are contrary to results obtained in the present study.

Although there was a reduction of SUN in the wheat fed group as compared to the casein fed group of rats, no conclusion can be made about the mechanism. The possible explanation is that the urea excretion of wheat fed rats may be higher than casein fed rats, as suggested by Kiriyama *et al*. (113).

Chapter III

The Effect Of Diet High In Wheat Bread On Various Parameters Of The Blood And Urine In Human Subjects

Introduction

The level of urea in the blood is used as an index of kidney function. Any unexplained increase in the blood urea level represents a potential derangement in kidney function.

One of the dietary factors that is associated with an increase in blood urea level is the protein intake. The more protein consumed, the higher the blood urea. Work in this area has involved a mixture of proteins, with the consequence that no distinction has been made among proteins as far as their effect on blood urea is concerned.

Michigan State University bread study, a decade ago, indicated that subjects had normal serum urea nitrogen (SUN) levels while they consumed typical American diets which provided them with 70 g protein per day. When this dietary regimen was changed to one providing the same amount of protein, but all of it from plant sources, the SUN was markedly reduced (5). The validity of the initially observed reduction in blood urea level when a wheat diet was consumed has been confirmed over the intervening years by short-term studies in our laboratory.

In order to further explore this phenomenon, experiments have been designed both for rats and humans in which comparisons have been made between high wheat and normal diets. The main objectives of the experiments were to see (1) whether a high wheat diet has an effect on blood urea nitrogen level, and (2) whether the changes in SUN are related to the acid-base balance of the subject.

Experimental Procedure

Plan of Study

Subjects: Twelve normal, young college males were the subjects in this study. Each subject received a physical examination, with special attention being given to the condition of his kidneys. However, four of the twelve subjects could not participate for the entire period of the study, as their schedules did not permit them to do so. Four of the subjects volunteered to continue on the study for dietary periods V, and VI, which were repetitions of periods I and IV during which the regular and the bread diets, respectively, were consumed. This was done to check the repeatability of the effects observed during these respective periods.

All eight subjects were in good health as shown by their physical examinations, urine analyses, standard hematological and clinical tests performed at the student health center. The subjects were asked to sign the consent form and answer a questionnaire (Appendices B and C) before the start of the experiment.

Duration of Study: Fifty-four days--October 12 through December 5, 1975 (Appendix D).

Dietary Schedule: The entire 54-day study was divided into six metabolic periods of varying lengths. During the study, the daily protein intake by the subjects was limited to 70 g. The subjects were permitted to adjust their caloric intake to maintain body weight using protein-free foods, thus maintaining a constant protein intake. The foods were served in Olin Health Center cafeteria at Michigan State University.

The schedule for the six dietary periods was:

Period I: October 13-31, 1975. A diet providing 70 g protein from animal and plant sources was consumed for 19 days. The menu for each day was selected from foods prepared in the Olin Health Center cafeteria. Each subject consumed the basic menu providing 70 g of protein and approximately 3000 calories. For extra calories, they were permitted to consume butter, honey, jelly or other protein-free foods. A duplicate of all the "core" foods served the subjects was saved for analysis (Table 21); that was done for every day of the study.

Period II: November 1-5, 1975. The dietary program for Period I was continued. In addition, each subject ingested 10 g of sodium bicarbonate in three doses: 2.5 g at breakfast, 3.5 g at lunch, and 4.0 g at dinner, in a glass of water. Sodium bicarbonate was included in the diet program to produce an alkaline condition in the body.

Period III: November 6-7, 1975. Period III was a transition between the mixed diet and the wheat diet (Period IV). During these two days, the subjects were given the opportunity to become adjusted to eating increasing amounts of wheat products. It requires only a few days for appropriate alterations to occur in the gastrointestinal tract. Once that has occurred, the subjects are able to consume large amounts of bread without any difficulty. No sodium bicarbonate was given in Period III.

Period IV: November 8-22, 1975. For 15 days, the subjects consumed diets providing them with 70 g of protein per day with about 90% of it coming from wheat and much of it in the form of white bread. There was no animal protein in these diets except a very minute amount of whey protein which came from the butter. That amounted to about 0.6 g per subject per day. For adjusting the caloric requirement, the subjects again were permitted the same protein-free foods used in the foregoing periods.

Period V: November 23-29, 1975. The diets used during this 7-day period were similar to those used in Period I.

Period VI: November 30-December 5, 1975. This 6-day period was similar to Period IV.

Periods V and VI were repetitions of Periods I and IV during which the regular and bread diets, respectively were consumed. This was done to check the repeatability of effects produced during Period I (Regular Diet) and Period IV (Bread Diet).

Sample Collection

Food Samples: As mentioned previously, during the entire study, an exact replica of the food in the core menu served the subjects was collected. These diet samples were analyzed for their nitrogen content to secure the exact nitrogen intake of the subjects.

Blood Samples, 24-hour Urine and Stools: These were collected in the morning before breakfast at different dates during the study as listed in the calendar of events as shown in Appendix D. The method of collection was the same as described in earlier chapters.

Analysis

Diets

All subjects during the entire period of the study ate all their food at the Olin Health Center cafeteria at Michigan State University. The diets were adjusted to provide each subject with the same amount of protein and an intake of calories which maintained their body weights (Table 21). Since the subjects differed in their physical activities, caloric requirements were adjusted by means of candies and protein-free foods. Amounts of the latter that were consumed by each subject, as well as weight of the food in the core diets were recorded each day for all subjects. These data permitted estimating the caloric intakes of each subject. The nitrogen content of each diet was determined by means of the semi-macro-Kjeldahl analysis (197). The results are listed in Table 21.

Blood Samples

Initially, blood chemistries were performed either by a commercial laboratory (Laboratory of Clinical Medicine) or the Student Health Center. The blood samples

TABLE 21.--Daily protein, fat and calories in diets used for human subjects throughout the entire study.

Date	Protein (g) (By Analysis)	Fat (g) (Estimated)	Calories (Estimated)
Control (Period I)			
October 13	71	95	2626
October 14	69	95	2435
October 15	69	94	2445
October 16	74	89	2723
October 17	72	114	2762
October 18	70	92	2815
October 19	71	104	2787
October 20	72	93	2779
October 21	69	120	2756
October 22	72	115	2718
October 23	71	107	2968
October 24	70	131	2702
October 25	71	108	2783
October 26	70	104	2798
October 27	70	94	2983
October 28	69	109	3038
October 29	71	122	3081
October 30	71	104	3021
October 31	69	90	3061
Average	70±1	104±12	2804±187

TABLE 21.--(cont'd.).

$NaHCO_3$ (Period II)			
November 1	68	116	2980
November 2	69	112	2968
November 3	69	100	2973
November 4	69	131	3047
November 5	71	111	2934
November 6	70	107	3159
Average	69±1	112±10	3010±82
Bread (Period IV)			
November 7	71	94	3297
November 8	69	83	2996
November 9	69	80	3210
November 10	72	78	2926
November 11	70	51	2813
November 12	70	71	3093
November 13	71	82	3042
November 14	70	67	2964
November 15	71	81	2944
November 16	69	81	3044
November 17	70	101	3147
November 18	67	64	2925
November 19	69	85	3002
November 20	68	86	3113
November 21	67	96	3033
November 22	67	63	3007
November 23	71	132	3098
Average	69±1	90±22	3037±119

TABLE 21.--(cont'd.).

Control (Period V)			
November 24	69	109	3097
November 25	69	126	2961
November 26	69	109	3097
November 27	72	101	3040
November 28	71	126	2961
November 29	68	126	2961
Average	69±1	116±11	3019±67
Bread (Period VI)			
November 30	70	74	3166
December 1	68	92	3200
December 2	69	74	3166
December 3	69	92	3200
December 4	68	74	3166
December 5	70	75	3369
Average	69±1	80±9	3155±122

(anticubital vein) were drawn at the Student Health Center where the blood pH, hemoglobin, hematocrit, plasma HCO_3^- and total CO_2 were determined. The sera were sent to the commercial laboratory for analysis of the other blood constituents, as described in Chapters I, Part III.

Urine

Urines were analyzed for titratable acidity and pH as soon as they were brought to the laboratory. The 24-hour urine volumes were recorded every time urine was collected. The procedures are described in Chapter I, Part II.

Results

Serum Urea Nitrogen (SUN)

The SUN values may have slightly decreased when the subjects were given 10 g/day of $NaHCO_3$ along with their control diet; however this change was not statistically significant. When the subjects were transferred to the wheat bread diet (Table 22), at first the SUN returned to the control values, but after 4 days the SUN may have decreased; but the change is not statistically significant. Four of eight subjects who continued for a longer period (Period V and VI, which were repetitions of Periods I and IV) showed a possible increase in the SUN levels 6 days after their diets were changed to the typical American pattern; however, this change was not statistically significant. When those subjects were again fed the high wheat diet, the SUN seemed to decrease after three days and remained there throughout the remainder of the period; however these changes were not statistically significant. It should be pointed out that even though the above changes were not statistically significant, the observation that the changes were repeatable adds to the credibility.

Serum Creatining and Serum Uric Acid

The serum creatinine (Table 23) of the subjects showed very slight fluctuations during the entire period of the study, but there was no definite change during any part of the dietary regimen. At all times, these values remained within normal limits (Table 23). Similarly, uric acid (Table 24) fluctuated to a very small extent which was of no apparent physiological or biochemical significance, since the variations were within normal limits.

Serum Protein and Albumin

Serum protein and albumin values remained relatively constant within the normal range over the entire course of the study (Tables 25,26).

Hemoglobin and Hematocrit

No alterations were observed throughout the entire period of study as far as these parameters were concerned. They remained well within normal limits (Tables 27,28).

pH, Bicarbonate, Carbon Dioxide and Serum Electrolytes

Similarly, blood pH, plasma HCO_3^- and CO_2 remained essentially unchanged on all the dietary regimens (Tables 29-31). The serum electrolytes, Na^+, K^+, Ca^{++} and phosphorus remained constant (Tables 32-35). The constancy of these parameters occurred despite the large amount of $NaHCO_3$ given the subjects for six days during Period II.

Urinary Acidity

The pH of the urine (Table 36) increased from 6.1 ± 0.2 on the control diet to 7.5 ± 0.2 when the subjects consumed 10 g of $NaHCO_3$ with the control diet. The pH of the urine dropped to 6.0 ± 0.2 when the subjects were fed the high wheat diets. On the other hand, total titratable acidity of the urine was 34.0 ± 2.5 mEq/day when the subjects were fed the control diet. When the subjects were on the wheat diet, the titratable acidity statistically significantly decreased, indicating that their body fluids became more alkaline as evidenced by the fact that the urine titratable acid excretion dropped to 13.8 ± 2.9 mEq/day.

Cholesterol and Glucose

The serum cholesterol value decreased slightly in each individual while he ate the bread diet. There was an increase in serum cholesterol when four out of the eight subjects were fed the control diet (Period V) but it dropped from 181 to 154 mg/dl during the bread diet which is not significant statistically (Table 37).

Similar trends in serum glucose concentration which were not statistically significant were observed (Table 38). Since no inhibitor of glucose metabolism was added to the blood when it was collected, the serum glucose analysis was probably underestimated.

TABLE 22.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum urea nitrogen levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SUN mg/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	15.0	14.0	12.0	15.0	14.0	14.0	13.0	14.0	17.0	15.0	13.0	14.0
R.G.	15.0	12.0	12.0	10.0	10.0	12.0	12.0					
E.K.	11.0	12.0	9.0	12.0	10.0	12.0	11.0					
T.L.	17.0	11.0	11.0	10.0	9.0	11.0	10.0	12.0	14.0	12.0	10.0	10.0
E.L.	14.0	13.0	12.0	15.0	11.0	13.0	14.0					
K.S.	13.0	12.0	10.0	12.0	10.0	12.0	9.0	11.0	13.0	11.0	11.0	12.0
J.U.	13.0	12.0	10.0	11.0	9.0	10.0	10.0	10.0	11.0	11.0	10.0	8.0
B.V.	16.0	12.0	11.0	12.0	10.0	11.0	13.0					
Average	14.4	12.2	10.9	12.1	10.3	11.9	11.5	11.7	13.7	12.2	11.0	11.0
S.D.	±1.9	±.8	±1.1	±1.9	±1.5	±1.2	±1.7	±1.7	±2.2	±1.8	±1.4	±2.5

TABLE 23.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum creatinine levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM CREATININE mg/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	1.0	1.1	1.0	1.1	1.1	1.0	1.0	1.0	1.1	1.1	1.1	1.0
R.G.	0.9	1.0	0.8	1.1	1.1	1.0	0.9					
E.K.	1.0	0.9	1.0	1.0	1.0	0.8	0.8					
T.L.	1.0	1.0	1.1	1.0	1.0	1.0	0.9	0.9	1.0	0.9	1.0	1.0
E.L.	1.1	1.2	1.2	1.3	1.0	1.1	1.0					
K.S.	1.1	1.1	1.1	1.1	1.0	1.1	1.0	1.1	1.1	1.1	1.1	1.2
J.U.	1.0	1.0	1.1	1.0	1.0	1.0	0.9	1.0	1.0	1.0	0.9	1.0
B.V.	1.0	1.1	1.1	1.2	1.1	1.0	1.1					
Average S.D.	1.0 ±.07	1.0 ±.09	1.0 ±.10	1.10 ±.10	1.0 ±.05	1.0 ±.09	1.0 ±.09	1.0 ±.08	1.0 ±.05	1.0 ±.09	1.0 ±.09	1.0 ±.01

TABLE 24.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum uric acid levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	URIC ACID mg/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	4.4	6.8	5.2	4.7	5.5	5.8	5.1	5.4	5.4	5.6	5.2	5.6
R.G.	5.3	5.9	5.8	5.7	6.5	5.7	5.6					
E.K.	5.4	5.3	5.5	5.2	5.4	5.5	5.2					
T.L.	4.8	4.9	4.8	4.8	5.2	5.4	5.2	5.8	5.4	4.9	5.2	5.4
E.L.	6.1	5.9	5.5	5.8	6.0	6.0	6.3					
K.S.	6.8	6.6	5.8	6.1	6.8	7.7	8.1	7.2	6.9	5.9	6.9	6.9
J.U.	5.1	6.1	6.3	6.1	7.4	6.7	6.6	6.9	6.7	6.4	6.4	6.5
B.V.	6.4	5.4	6.0	6.3	6.1	5.7	6.2					
Average	5.5	5.8	5.6	5.5	6.1	6.0	5.9	6.3	6.0	5.7	5.9	6.1

TABLE 25.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum protein levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM PROTEIN g/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	8.6	7.5	7.0	7.2	7.3	7.1	7.4	7.4	7.2	7.1	7.1	7.2
R.G.	8.8	7.6	8.0	7.1	7.2	7.1	6.9					
E.K.	7.0	6.8	6.5	6.3	6.6	6.7	6.4					
T.L.	8.0	7.8	8.3	8.3	7.3	7.5	6.7	7.6	7.3	7.1	6.5	7.4
E.L.	7.4	7.3	8.1	7.1	7.6	7.2	6.7					
K.S.	7.8	7.4	6.7	7.3	7.3	8.0	8.1	7.5	6.8	6.7	7.4	7.5
J.U.	7.3	7.3	6.5	6.9	7.5	6.6	6.6	7.2	6.5	6.6	6.5	7.0
B.V.	7.7	7.8	7.3	7.3	7.6	7.1	7.2					
Average	7.8	7.4	6.9	7.0	7.3	7.0	6.8	7.4	6.0	5.7	5.6	7.2

TABLE 26.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum albumin levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM ALBUMIN g/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	4.8	4.6	4.4	4.6	4.8	4.4	4.9	4.4	4.8	4.8	4.7	4.7
R.G.	4.7	4.5	4.0	4.4	4.4	4.2	4.4					
E.K.	4.5	4.4	4.1	4.5	4.5	4.5	4.5					
T.L.	4.8	4.6	4.5	4.6	4.5	4.6	4.6	4.4	4.6	4.7	4.2	4.7
E.L.	4.7	4.6	4.7	4.8	4.0	4.8	4.7					
K.S.	4.8	4.6	4.3	4.8	4.9	4.5	4.8	4.6	4.6	4.7	4.9	5.0
J.U.	4.7	4.6	4.2	4.7	4.9	4.4	4.6	4.4	4.5	4.7	4.6	4.7
B.V.	4.7	4.8	4.6	4.9	5.0	4.6	4.9					
Average	4.7	4.6	4.3	4.7	4.6	4.5	4.7	4.4	4.6	4.7	4.6	4.8

TABLE 27.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum hemoglobin levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM HEMOGLOBIN g/dl						
	I		II	IV			
Period	Control		$NaHCO_3$	Bread			
Subject/Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20
T.B.	13.3	13.2	13.0	13.0	13.2	12.6	13.3
R.G.	15.8	15.8	15.0	15.7	15.9	15.4	15.2
E.K.	15.3	15.1	15.5	15.0	15.3	15.5	15.4
T.L.	13.9	14.4	14.0	13.8	13.9	13.3	13.4
E.L.	15.2	14.9	15.6	15.2	15.9	15.3	15.2
K.S.	14.2	15.0	14.5	14.9	14.9	14.2	14.0
J.U.	15.4	15.0	14.9	14.9	15.2	14.5	14.3
B.V.	14.8	14.9	15.0	14.2	15.1	14.9	14.4
Average	14.7	14.8	14.7	14.6	14.9	14.5	14.4

TABLE 28.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on hematocrit levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study. All the values are expressed as volume %.

	HEMATOCRIT						
	I		II	IV			
Period	Control		$NaHCO_3$	Bread			
Subject/Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20
T.B.	39	38	38	38	37	36	38
R.G.	46	44	44	45	45	44	43
E.K.	44	43	44	43	43	44	43
T.L.	40	42	40	40	39	38	38
E.L.	44	43	45	44	45	44	43
K.S.	42	44	42	44	44	41	41
J.U.	43	43	43	42	43	41	40
B.V.	43	43	44	42	44	44	42
Average	43	42	42	42	42	41	41

TABLE 29.--Blood pH of the individuals fed isonitrogenous the same type of diet, typical omnivorous American diet + 10 g sodium bicarbonate per person per day and 'high bread' diet. The last values listed under each dietary period represent the sample collected at the end of that phase of the study.

Period	I Control		II $NaHCO_3$	IV Bread			
Subject/Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20
T.B.	7.35	7.35	7.35	7.32	7.34	7.39	7.34
R.G.	7.32	7.33	7.37	7.31	7.35	7.35	7.36
E.K.	7.33	7.34	7.36	7.37	7.32	7.33	7.33
T.L.	7.38	7.33	7.42	7.35	7.39	7.38	7.38
E.L.	7.36	7.31	7.32	7.37	7.27	7.32	7.37
K.S.	7.34	7.29	7.32	7.23	7.30	7.30	7.31
J.U.	7.36	7.32	7.34	7.34	7.33	7.39	7.35
B.V.	7.31	7.25	7.38	7.25	7.29	7.34	7.30
Average	7.3	7.3	7.3	7.3	7.3	7.3	7.3

TABLE 30.--Effect of typical American diet, the same type of diet + 10 g $NaHCO_3$ per person per day, and 'high bread' diet on plasma bicarbonate of the individuals. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

PLASMA BICARBONATE mM/L							
Period	I Control		II $NaHCO_3$	IV Bread Diet			
Subject/Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20
T.B.	28	30	32	31	32	31	31
R.G.	27	31	30	32	34	32	33
E.K.	27	25	36	30	31	31	29
T.L.	29	27	31	32	31	31	34
E.L.	29	24	30	25	25	30	31
K.S.	25	27	31	31	31	31	32
J.U.	29	28	31	30	33	35	32
B.V.	24	26	29	29	29	28	31
Average	27	27	31	30	30	31	31

TABLE 31.--Effect of typical American diet, the same type of diet + 10 g $NaHCO_3$ per person per day, and 'high bread' diet on plasma carbon dioxide of the individuals. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

PLASMA CARBON DIOXIDE mM/L							
Period	I Control		II $NaHCO_3$	IV Bread			
Subject/Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20
T.B.	30	32	20	29	31	27	27
R.G.	29	32	26	30	30	28	29
E.K.	29	27	25	27	28	27	27
T.L.	30	29	28	30	29	27	30
E.L.	30	27	28	22	23	24	29
K.S.	27	29	27	28	28	27	29
J.U.	30	30	27	27	30	29	30
B.V.	26	27	27	27	27	25	27
Average	28	29	27	27	28	26	28

TABLE 32.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum sodium levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM SODIUM mEq/L											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	143	144	139	143	143	145	142	140	140	142	140	142
R.G.	142	141	138	143	141	141	142					
E.K.	142	144	138	144	143	142	142					
T.L.	144	145	139	144	140	141	141	140	138	140	140	141
E.L.	140	139	139	140	141	139	140					
K.S.	144	146	137	143	141	141	142	141	140	141	141	143
J.U.	142	141	137	142	142	140	140	139	139	139	139	141
B.V.	143	142	138	145	142	140	143					
Average	142.5	142.8	138.1	143	141.6	141.1	141.5	140.0	139.2	140.5	140.0	141.7

TABLE 33.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum potassium levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM POTASSIUM mEq/L											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	4.4	4.5	4.2	4.2	4.4	3.9	4.6	4.5	4.5	4.2	4.3	4.0
R.G.	3.4	3.3	3.6	3.6	3.5	3.5	3.7					
E.K.	4.1	4.2	4.7	4.0	3.7	3.9	3.9					
T.L.	4.1	4.0	4.3	3.9	3.8	3.6	4.4	4.4	4.3	3.9	3.8	3.7
E.L.	4.3	4.2	4.4	3.7	3.9	4.2	4.5					
K.S.	4.2	4.4	4.3	4.0	4.7	4.3	4.6	4.4	4.4	4.2	4.1	4.2
J.U.	4.0	3.9	3.8	3.7	3.8	3.6	4.2	4.5	3.9	4.6	3.9	4.2
B.V.	3.9	3.8	4.0	4.6	4.5	3.8	4.2					
Average	4.0	4.0	4.0	3.9	4.0	3.8	4.2	4.4	4.2	4.2	4.0	4.0

TABLE 34.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum calcium levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM CALCIUM mg/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	10.2	9.9	9.8	10.0	10.0	9.9	10.4	10.2	10.3	10.0	9.6	10.1
R.G.	9.4	9.6	8.8	9.4	9.1	9.1	9.5					
E.K.	10.0	9.6	9.2	9.6	9.2	9.6	9.6					
T.L.	10.1	9.7	9.6	10.0	9.6	9.9	10.1	10.1	10.0	9.8	9.4	9.9
E.L.	9.3	9.9	10.0	10.0	9.2	10.1	10.2					
K.S.	10.3	9.7	9.6	10.2	10.0	9.9	10.3	10.5	9.9	10.0	10.2	10.3
J.U.	9.9	9.7	9.2	10.1	9.8	9.6	9.9	10.3	9.8	10.0	9.6	10.2
B.V.	9.8	10.1	9.8	10.4	10.3	9.8	10.6					
Average	9.9	9.8	9.5	9.9	9.6	9.7	10.1	10.3	10.0	9.9	9.7	10.1

TABLE 35.-Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum phosphorus levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	SERUM PHOSPHORUS mg/dl											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	3.8	3.5	3.2	3.5	4.3	3.3	3.6	3.6	3.9	3.7	3.8	3.4
R.G.	3.3	2.8	3.5	2.7	3.0	3.1	2.8					
E.K.	4.4	4.0	3.8	4.1	4.0	4.0	4.2					
T.L.	3.6	3.0	2.6	2.8	3.1	2.9	3.6	3.0	3.3	3.0	3.4	3.0
E.L.	4.8	4.5	4.4	4.5	4.7	4.4	4.5					
K.S.	4.8	4.2	3.9	4.2	4.0	4.7	4.7	4.7	4.5	4.5	5.2	5.6
J.U.	3.4	3.5	3.6	3.8	3.8	3.2	3.3	3.2	3.2	3.6	3.3	2.8
B.V.	4.5	4.8	3.9	4.6	4.3	4.1	4.4					
Average	4.0	3.8	3.6	3.8	3.9	3.7	3.9	3.6	3.7	3.7	3.9	3.7

TABLE 36.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on the excretion of titratable acid of the individuals as reflected by urinary acidity. The values given are the averages of the last 3 days of each dietary period.

Subject	Urine pH			Titratable Acid Excretion mEq/day[xx]		
	I Control	II $NaHCO_3$	IV Bread	I Control	II $NaHCO_3$	IV Bread
T.B.	5.96	7.29	5.83	39.6	14.3	15.7
R.G.	6.45	7.72	5.92	21.8	11.8	12.1
E.K.	5.98	7.32	5.59	43.0	7.5	16.7
T.L.	6.20	7.38	6.22	32.3	12.3	10.3
E.L.	6.27	7.51	6.05	27.2	11.4	16.7
K.S.	5.97	7.65	6.14	35.0	10.7	14.3
J.U.	6.28	7.55	6.24	39.2	12.6	12.8
B.V.	6.07	7.55	6.11	33.9	12.6	11.7
Average	6.1±0.1[x]	7.5±0.1	6.0±0.2	34.0±6.9	11.7±1.8	13.8±2.9

[x]Standard deviations are given all average values

[xx]Titratable acid excretion is significantly ($P<0.05$) low on bread diet as compared to control diet

TABLE 37.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum cholesterol levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	CHOLESTEROL (mg/dl)											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	165	172	179	185	188	183	192	191	193	191	170	174
R.G.	207	230	208	183	184	200	203					
E.K.	160	170	161	151	157	165	156					
T.L.	185	185	178	169	173	191	182	206	206	194	157	156
E.L.	173	150	157	151	164	150	152					
K.S.	160	140	139	154	153	141	147	168	153	148	148	151
J.U.	150	145	137	145	158	157	141	160	150	156	132	138
B.V.	170	150	150	141	150	158	141					
Average	172	167	163	159	165	168	164	181	175	172	151	154

TABLE 38.--Effect of typical omnivorous American diet, the same type of diet + 10 g sodium bicarbonate per person per day, and 'high bread' diet on serum glucose levels of human subjects. The last values listed under each dietary period represent the samples collected at the end of that phase of the study.

	GLUCOSE (mg/dl)											
	I		II	IV				V		VI		
Period	Control		$NaHCO_3$	Bread				Control		Bread		
Subject/ Date	10-15	10-31	11-6	11-10	11-14	11-18	11-20	11-25	11-29	12-1	12-3	12-6
T.B.	75	77	74	76	77	91	78	76	72	78	77	63
R.G.	85	75	84	78	82	85	78					
E.K.	85	70	70	65	75	77	70					
T.L.	80	73	77	70	78	83	72	71	71	82	80	70
E.L.	92	80	71	75	68	82	73					
K.S.	70	67	73	73	69	79	76	72	71	73	74	58
J.U.	80	67	66	63	81	78	69	69	71	62	68	62
B.V.	85	82	79	80	97	91	84					
Average	81	74	74	72	78	83	75	72	71	73	75	63

TABLE 39.--Average body weight of the individuals fed various isonitrogenous diets.

Subjects	I Control		II $NaHCO_3$	IV Bread	
	10-15	10-31	11-3	11-9	11-17
T.B.	163	161	160	161.5	162.5
R.G.	125	125	126	126	129
E.K.	157	158	158	158	157
T.L.	160	161	161	162	163
E.L.	155	151.5	155	151	152
K.S.	178.5	173	173	174	174
J.U.	144	141.5	140	141	142
B.V.	164	159	159	159	159
Average	155±15[x]	143±14	154±14	154±14	154±13

[x]Standard deviations are given for all average values.

TABLE 40.--Urinary volume in milliliters of the individuals. Average of the last 3 days of each dietary period.

	ml/day		
	I	II	IV
Subjects	Control	$NaHCO_3$	Bread
T.B.	988	1100	910
R.G.	1280	1005	983
E.K.	1591	2173	1896
T.L.	1425	1591	1550
E.L.	996	1175	1335
K.S.	1145	1181	1553
J.U.	1326	1396	1501
B.V.	875	1075	823
Average	1203±245[x]	1337±387	1318±378

[x]Standard deviations are given for all average values.

Discussion

In order to lessen the work of diseased kidneys, limitation of the protein intake was, for many years, the chief aim in therapy, so that the patient was deprived of protein to a very great extent and was in many cases subjected to more or less drastic regimens of starvation. Realizing the unsatisfactory nature of the older methods of treatment, the present work was undertaken to explore the effect on urea metabolism of diets in which the sole source of protein was from plants. The patient should not, therefore, be made needlessly uncomfortable and unhappy by useless dietetic and other restrictions. The present work consisted of two studies, one with humans and one with rats. The purpose was to examine the effects diets high in wheat have upon normal individuals' serum urea levels, together with observations upon various parameters of the blood and urine.

Since 1964, when the MSU bread study was carried out, there was some question about the validity of the reduction in blood urea nitrogen observed when normal young college students consumed a diet in which 90 to 95% of their protein came from wheat (5). The subject's SUN levels were reduced when compared to the levels these subjects had when consuming isonitrogenous diets in the control period. Another report (18) which appeared in the literature as early as 1931, reported that in their study with advance chronic interstitial nephritis, the proteins of vegetable origin were better tolerated by their renal patients. They further claimed that in addition to the general improvement of kidney functions, the blood urea nitrogen levels dropped to nearly normal levels from the previous elevated levels. In that study, only the kind, and not the amount of protein was varied. The protein of the basic diet was derived from vegetable origin. Another report by Kies and Fox (107) demonstrated a more than 50% decrease in blood urea levels in their normal subjects when they shifted from a control to a wheat diet.

In the present study, the protein intake of the subjects for the entire period averaged 70 g per day. Since body weight maintenance was required, supplemental protein-free foods were provided each subject. The supplement was adjusted according to the subject's body weight as recorded each week (Table 39).

The serum urea levels may have decreased gradually. There might have been a drop in SUN when subjects were on the control diet and were ingesting 10 g/day of $NaHCO_3$. A further slight drop might have occurred when the subjects were fed diets in which the major protein source was wheat. Also a possible decrease in SUN was observed when the subjects were on the control diet (Period I) for 15 days. This reduction over that period represents the adjustments brought about by the decreased protein intake when the subjects began eating the control diet. Previous experience with groups of comparable college men, suggests their daily protein intake is from 100 to 150 g per day, when they consumed an unrestricted diet. When limited to a dietary intake of 70 g of protein per day, a period of more than two weeks is required for the establishment of an equilibrium state in the serum urea levels (5).

That plant proteins when properly combined in the diet may be as effective for growth as animal protein, has been shown by the work of Kofryani and Muller-Weeker (198). They emphasized that to secure valid results, the experiment must be sufficiently long to establish an equilibrium state, which is longer than the one week, that had been used for many experiments. One week may be sufficient for establishing equilibrium in small animals, like rats, but is not long enough for humans. They reported that the nitrogen balance during the first part of the test period is strongly negative and may continue that way for up to 11 days. It was only during the subsequent 17 days that the organism attained an equilibrium between protein requirement and protein consumption. Only values secured during the steady state should be used for calculating the over-all effects on the body.

Our earlier preliminary results suggested that for maximum reduction in the SUN levels, normal subjects may have to be maintained on the bread diet for not more than 15 days. But from the present study and the results of other investigators, it appears that the longer time required for the maximum reduction in SUN levels may be important from a physiological standpoint. The changes in the renal and extra-renal function which effect urea metabolism during a more prolonged consumption of the wheat diet, may indicate more profound and more permanent alterations in physiological functions than might occur in a short time interval. This has become evident from the observations made in the present study which was of shorter duration.

On the basis of previous work, it is generally believed that the only dietary factor affecting the blood urea nitrogen level in normal subjects is the amount of protein consumed. Prior to the work done by Bolourchi et al. (5) in our laboratory, there was no theoretical reason for doubting the concept that the amount of protein in the diet determined the level of urea in the blood which presumably reflected the amount of urea formed. However, no thought was given to the possibility that, in normal individuals, the kidney function could be influenced by such a minor factor as the consumption of a vegetarian diet. There are very few pertinent studies in the literature and most of them are incomplete in one respect or another or present conflicting results. Taylor et al. (114) demonstrated that there is an inverse relationship between the net protein utilization and serum urea concentration. They reported that in adult men, the biological value of a protein is insignificantly and negatively correlated with the SUN concentration secured when the wheat protein is the major source of dietary nitrogen. Again, questions arise as to whether these investigators have properly considered the duration of the experimental period. Their complete dietary study extended for only 15 days. Such a short period has been criticized (1,198). In the present experiment, such a short period was not long enough to observe statistically significant changes in SUN when diets high in wheat bread were consumed. Another factor in their study which may become important, was the use of synthetic formula diets. In their study, they incorporated wheat gluten instead of wheat flour into a liquid formula. The mineral mixture which they added to their liquid diet appears to have been one factor causing the elevation of the SUN levels. Elevated SUN levels have been shown in Part II of this thesis, to be lowered in 3 out of 4 subjects when the diet was supplemented with 10 g of $NaHCO_3$.

Several investigators have recommended the use of a low protein diet for kidney patients as a means of reducing the production of urea (199-202). According to these workers, at any given level of renal function, the degree of uremia is in part related to the protein intake. By reducing the protein intake to minimum levels, it is possible to reduce the blood urea level, whereas, if standard diets were used, severe uremic symptoms would develop.

It was only recently that it was suggested that the imposition of a low protein intake for patients in chronic renal failure introduces in the long term, more trouble than it is really worth (115). The author further suggested that in compensation for a low protein intake, a patient is obliged to eat supernormal amounts of carbohydrates and fat which increase triglyceride formation, thus causing hyperlipidemia. Young and Parson (116) also suggested that low protein diets despite a high intake of calories, are subject to hazards and reported that these should be avoided. This is especially so since uremic patients have been reported to have disorders of carbohydrate and lipid metabolism which may be aggravated by low protein and high caloric diets (203-206).

The data in Table 22 show a possible reduction with time in the serum urea levels from the initiation of the study through the period when a sodium bicarbonate supplement was given. For six days, each subject received 10 g / day of sodium bicarbonate which was taken with breakfast, lunch and dinner. The sodium bicarbonate was given since a number of reports indicated that this alkalinizing agent lowers the serum urea level not only in patients who have certain kidney disturbances but in normal individuals, as well. Although these data show a reduction in urea levels for the blood secured at the end of the sodium bicarbonate period, its significance is open to question.

On the basis of earlier, preliminary studies performed with graduate students, it appeared that the blood urea level decreased within a matter of a few days when the subjects changed from a normal, omnivorous diet to one providing most of the protein from wheat. No controls or checks were used in those studies other than the student's estimate of his or her protein intake, both during the control and "bread" periods. For that reason, it was assumed that the blood urea level should show a dramatic reduction shortly after the subjects were shifted from a control diet containing a normal amount of animal protein to one containing no animal protein and with most of that nutrient coming from wheat.

When the subjects in the present study were fed a diet providing most of their protein from wheat (Period IV), there was some, but only a minimal reduction in the serum urea levels after many days. The reduction in the urea levels was much smaller than anticipated, despite our efforts to rigidly control all aspects of the study. Throughout its duration, the diets were controlled to maintain each subjects' protein intake at 70 g per day.

A number of explanations were considered for the slight response in the urea level when the "bread" diet was fed. The explanation that appeared most plausible was that the bread might have been made with the usual amount of skim milk powder. That possibility was entertained since bread for this study was made by a different baker than the one from whom we purchased the bread used in our previous studies. After the death of our first baker, we located another bakery where we were assured that the bread would be made to our specifications. Since the blood urea changes in the subjects fed that bread were not the same as in the earlier study, we felt the bread probably contained skim milk powder.

To check whether any skim milk powder, that might have been in the bread, was responsible for the observed results, four subjects were persuaded to continue on the study for an additional period. During the first week of that period, diets were served which provided a daily average of 69 g protein for each subject. As much of the protein as possible, in the period, came from animal sources. For the next six days, the diets contained no animal protein and practically all the protein came from the bread baked by Mrs. Mickelsen who undertook that operation to make cartain the bread was free of animal products.

The results of that part of the study (Period VI) indicated that the blood urea level for all subjects responded to the dietary changes in the same way (Table 22). The time required for an equilibrium state may be more than six days even when there is no change in the level, but a drastic alteration in the nature of the dietary protein. This is especially pronounced when the subjects changed from their regular type diets to those containing large amounts of wheat products. Finally, these data suggest that the level of blood urea is characteristic of the subject and he retains his relative standing on that score regardless of the dietary changes.

While the subjects consumed the "bread" diet in Period IV, their blood urea levels fluctuated somewhat, but in 3 out of 4 cases were lower than what they were when the second control diet (Period V) was fed (Table 22). For all subjects, there was an increase in blood urea levels when the control diet (Period V) containing a large proportion of the protein from animal sources was fed. The latter diet was developed so that 60% of the protein was from animal sources. Again, the data suggest that a 6-day period is not adequate for the adjustment of the blood urea level to the nature of the dietary protein. This must mean that a more profound alteration in the body's physiological response is occuring than adjustment in the levels of enzymes involved in the metabolism of amino acids or in the formation and catabolism of urea.

Although the "bread" diet produced a possible reduction in the urea level of the blood, it had no measurable effect on the serum creatinine levels. One possible implication of this observation is that the change brought about in the urea levels of the blood represents alterations in some metabolic process other than kidney function. Additional studies will be necessary to evaluate that hypothesis.

The constancy of the hemoglobin levels throughout the entire study (Table 27) indicates that the inclusion of large amounts of wheat products in the diet has no adverse effect on the absorption of iron from the intestine. Since there was no reduction in the hemoglobin levels, these data suggest that, for young men, an adequate intake of enriched bread and other enriched wheat products should provide sufficient iron for hemoglobin formation. One might propose, on the basis of these observations, that to improve the iron status of the American public, nutrition education efforts might better be directed toward increasing the consumption of enriched cereal products.

One additional comment about the hemoglobin levels is that for normal individuals, the level appears to be characteristic of that person (Table 27). Those who have a relatively low level appear to maintain that for long periods, providing something doesn't happen, such as illness, weight loss, blood donation, etc., to influence the hemoglobin concentration. There does not appear to be any valid indication as to why some individuals have low levels whereas others have higher levels. Both subjects with the lowest levels of hemoglobin were physically active and were in good condition. The results of their physical examination did not indicate anything but an excellent health condition for both.

The serum albumin level is frequently used as an index of protein nutriture. If, for any reason, the dietary protein intake is not adequate to meet the needs of the body for the essential amino acids, a reduction usually occurs in the level of serum albumin. Since there was no change in the level of serum albumin through the course of the study (Table 26), it would appear that the alterations in blood urea levels associated with the changes in diet were not the result of any abnormalities in the protein nutritional status of the subjects.

There were no prominent changes in serum glucose levels during the first five weeks of the study (Table 38). The only change that merits any comment is the possible reduction in glucose levels in the blood secured at the end of the study. Those values for the four subjects who remained with the study were lower than most of their preceding values. That was especially true for the samples secured at the end of the rigidly controlled bread period. It may be that the possible reduction in blood glucose levels for these subjects represents an improved carbohydrate metabolism. The reason for suggesting that possibility is the report from two separate groups of investigators (207,208). These investigators reported a reduction in maximum blood sugar levels following a meal when the ordinary diabetic diet was supplemented with bran. Although bread has very little fiber, as measured by the AOAC technique, it does produce changes in the lower gastrointestinal contents comparable to those observed when the dietary fiber level is increased. For that reason, the increased use of ordinary bread may be an important factor in maintaining a healthful carbohydrate metabolism and possibly preventing the development of diabetes among those individuals who are genetically predisposed to the condition.

Our observations as described in this thesis and the observations made by Lyon and associates (17,18) suggest that blood urea levels are influenced by the acid-base conditions of the individuals. These results raise the question as to whether the SUN might be influenced by the alkaline condition in the body resulting from the wheat diet. The consumption of diets high in wheat products seem to result in a slow reduction in the SUN levels. That, however, has also brought a change in the acid-base homeostasis of the individual. The change observed was in the titratable acidity, which dropped significantly when the subjects ate the wheat diets as compared to the control diets. This probably is due to a smaller intake of $SO_4^=$ and $PO_4^{\equiv}$, as the diets of plant origin are generally low in these constituents. Urinary pH remained unchanged, as were plasma HCO_3^- and blood pH. There were very slight fluctuations in the serum uric acid concentrations. These changes were within normal limits and were of no physiological significance.

In conclusion, a slight non-statistically significant reduction in SUN which was observed on high wheat diets may either be due to the alkalinizing effect of the diet or increased excretion of urea. There can be no definite explanation of the possible increase in SUN when four out of eight subjects repeated Periods I. Unfortunately, the titratable acidity of their urine was not determined. The suggestion made by others that diets high in wheat may reduce SUN can still be made, if a period longer than two weeks is given to the experiment.

SUMMARY AND CONCLUSIONS

The effect of different dietary protein on the acid-base homeostasis and serum urea nitrogen concentration was examined using healthy, adult male human subjects and adult male Sprague-Dawley rats. In Part I, the main objective was to examine the effect of ingestion of sodium bicarbonate upon serum urea nitrogen (SUN) and acid-base balance. In Part II, the effect of the nature and source of dietary protein fed isonitrogenously upon the acid-base balance and SUN concentration was examined.

Part I

In this part, two experiments were carried out with adult, male, healthy human subjects.

Experiment 1: A group of three individuals were fed typical American diets for five days. During the five day control period, these individuals consumed typical American diets with each person eating the same kind and same amount of food every day. This was followed by the experimental period which also lasted five days, when each subject consumed the same diet but supplemented with 15 grams of sodium bicarbonate, in three equal doses per day, after each meal for five days. The titratable acidity dropped from 22.6 mEq/day during the first phase to 17.4 mEq/day during the second phase. The SUN decreased significantly on the average from 16.7 mg/dl in the first phase to 12.7 mg/dl when $NaHCO_3$ was given. No changes were observed in creatinine, glucose or cholesterol concentrations of the serum throughout the study.

From these observations, it was concluded that $NaHCO_3$, which is an alkalinizing agent lowers the SUN in human subjects. This does not effect the creatinine, glucose or cholesterol levels of the serum. These data support the observations made by earlier workers.

Experiment 2: During this experiment, four, healthy male, adult human subjects consumed a wheat gluten liquid formula diet for seven days in the control phase. In the experimental phase the subjects consumed the same daily amount of this liquid diet plus 10 g of $NaHCO_3$ in three doses every day for seven days. During the control phase, the urine of these subjects was highly acid with a titratable acidity of 95.2 mEq/day. The urine in the experimental phase became more alkaline with a titratable acidity of 24.2 mEq/day. The serum urea nitrogen in three of the four subjects decreased when the bicarbonate was given while in one case, there was an increase. There was no essential change in serum creatinine, sodium and potassium.

In conclusion, it may be suggested that the wheat gluten, liquid diet used in the control phase of the study, was an acidifying diet. When the acidotic condition was corrected with the ingestion of $NaHCO_3$, the SUN dropped but not significantly. It can be concluded from these data that any changes in acid-base homeostasis of the human subject has an effect on SUN. The decrease in SUN may possibly be due to (1) increased urea excretion and/or (2) less urea production.

Part II

To examine the effect of various kinds of protein fed isonitrogenously on the acid-base balance and SUN, four experiments were conducted. Three experiments were carried out with human subjects and one with rats.

Experiment 1 and 2: The first two experiments were exactly the same, but performed at two different times, first in February, 1975, and the second in October, 1975. In both experiments, nine (three in the first and six in the second) healthy male, adult individuals were fed a typical omnivorous American diet during a seven day control period. In the following seven day experimental period, the subjects consumed isonitrogenous and isocaloric diets, in which the primary sources of protein was from potatoes. Blood and urine samples were collected for the analyses of various constituents. The titratable acidity of the 24 hour urine was 20.7 mEq/day at the end of the control period and it dropped to 10.6 mEq/day at the end of the experimental period. Associated with that reduction in acidosis, the SUN also decreased from 13.3 mg/dl to 7.0 mg/dl in the first experiment and from 14.8 mg/dl to 10.3 mg/dl in the second experiment.

The above data suggest that the potato diet was an alkalinizing agent which lowered the SUN significantly. Thus, vegetarian diets which are basic in nature lower the SUN.

Experiment 3: This experiment was carried out with two groups of male, adult Sprague-Dawley rats. One group was fed a casein ration and the other, a wheat ration. Both rations were isonitrogenous and contained 10% protein. The rats were fed these rations ad lib for four weeks. At the end of the four weeks, blood samples were secured by cardiac puncture. Thereafter, the rats were pair-fed and after four weeks of such feeding, blood samples were again drawn by cardiac puncture. The SUN of the rats fed the casein diets at the end of the ad libitum feeding was 13.9 mg/dl and 13.8 mg/dl during the paired feeding, while the 10% wheat protein fed group of rats had SUN levels 11.6 mg/dl and 11.5 mg/dl on ad libitum and pair feeding which was significantly ($P<0.05$) lower than that of the casein-fed rats.

The wheat ration lowered the SUN in the adult rats when compared with the values in the casein-fed rations. This may be due to increased urea excretion, as reported by other workers.

Experiment 4: In this experiment, eight healthy, adult male college students were the subjects. Prior to the study, each subject received a physical examination with special attention to the condition of the kidney as determined by their serum creatinine and SUN. The entire 54-day study was composed of six metabolic periods of varying length. During this study, the daily protein intake of the subjects was limited to 70 g. The subjects were permitted to adjust their caloric intake with protein-free foods to maintain body weight. The foods were served in the Olin Health Center cafeteria at Michigan State University. Periods I and IV during which typical American diets and the bread diet respectively were consumed, were repeated as Period V and VI. This was done to examine the consistency of the reduction in SUN observed during Period IV.

Fasting blood samples were secured at the Olin Health Center. 24-hour urines were collected by each individual on almost every day of the study.

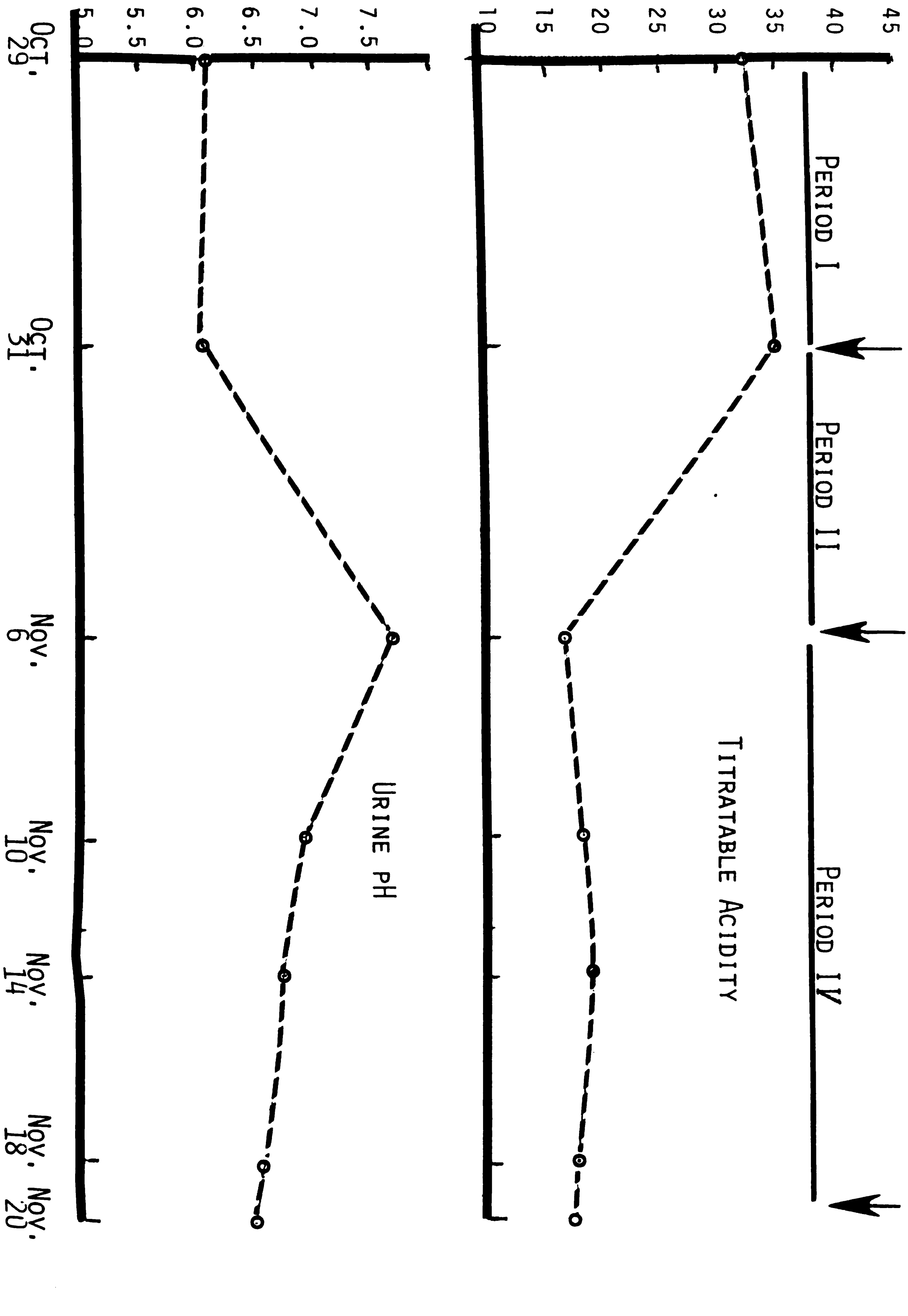
Titratable acidity of the urine indicated that the diet high in wheat fed to human subjects reduced the acidity of the urine. The average titratable acidity of the urine which was 34.0 mEq/day dropped to 13.8 mEq/day. Also a trend in the reduction of SUN was observed. There was no essential change in other constituents of the blood. When four of the eight subjects again consumed control diets high in animal protein for six days, their SUN increased. Following that, when they consumed the bread diet for another six days, their SUN was reduced. Unfortunately, the titratable acidity of the urine was not determined during these periods.

As an overall conclusion, it is suggested, on the basis of analytical data obtained from various experiments, that a change in acid-base balance of human subjects has a definite effect on SUN. Also proteins of plant origin, like potatoes and wheat flour, result in the formation of urine more alkaline than that produced when a diet high in animal protein is consumed. This is associated with a reduction in the SUN in humans. Furthermore, the SUN is lowered on high bread diets, if sufficient time is given to the experiment. Similar responses in SUN levels are seen in adult rats fed isonitrogenous diets in which the protein source is either casein or wheat. This reduction in SUN does not occur when weanling rats are used since wheat protein is not an adequate source of essential amino acids to support normal growth in these animals.

Another factor which has become important is the age of the rats. If weanling rats are used for this type of experiment, no reduction in SUN is observed, because of the greater nutritional requirements of the growing rats as compared with adult rats.

It can be suggested that extensive work is needed to examine the urea clearance, insulin levels of the plasma, glutamine stores, and degradation of urea in the gut.

Figure 5.--Average titratable acid excretion and urine pH of 8 subjects fed isocaloric and isonitrogenous (1) typical American diet; (2) same diet plus 10 g sodium bicarbonate, and (3) diet high in wheat bread.


PERIOD I
PERIOD II
PERIOD IV
TITRATABLE ACIDITY
URINE pH
45
40
35
30
25
20
15
10
7.5
7.0
6.5
6.0
5.5
5.0
OCT. 29
OCT. 31
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APPENDICES

Appendix A

COMPOSITION OF THE LIQUID FORMULA DIET USED BY MIT WORKERS

Ingredient	Amount/day in gms for a 70 kg man	Kcal
Liquid Formula:*		
Wheat Gluten	66.7	264
Maltose-Dextrin Mixture	170	680
Corn oil	130	1170
Lemon Juice	7.0	-
Vanilla	7.0	-
NaCl	1.0	-
Cellulose	2.0	-
K_2HPO_4	5.6	-
Water	550	-

Non Protein Energy Source, Composition

Ingredient	Composition/100 gm solution w/w	Amount for 70 kg man/day
Beverages	Soft drink powder (0.3) Sucrose (4.2) Carbohydrate (19.0)	1300 gm, supplying 1200 Kcal
Corn-Starch Dessert	Dessert (21.3) Sugar (3.9) Carbohydrate (3.95) Corn oil (2.6)	456 gm, supplying 630 Kcal
Protein-Free Cookies	Corn Starch (47) Sucrose (13.0) Salt (1.0) Carbohydrate (3.0)	Total of 8 cookies, supplying 1000 Kcal

*All the ingredients were obtained from the same source as reported by these investigators.

Appendix B

CONSENT FOR PARTICIPATION IN A STUDY TO EVALUATE THE EFFECT OF WHEAT FOODS ON BLOOD UREA LEVELS

I agree to serve as subject for a study to evaluate the effect of wheat foods on blood urea levels. I recognize that this will involve consuming a regular American type diet for 26 days and a vegetarian diet for 18 days consisting primarily of white bread, wheat products plus vegetables and fruits. I understand that the diets served me will provide 70 g protein and approximately 2,600 Kcal per day. If the caloric content of the diet is not sufficient to maintain my body weight, I will consume extra protein-free foods like purified butter, jam jelly, hard candies, etc. which will be made available to me. During the study, I will refrain from the consumption of any alcoholic beverages and eat only what is made available to me through the study dietitians in the Olin Health Center Cafeteria.

This study requires that I eat three meals each day for 44 days in the dining room of the Olin Health Center Cafeteria. I will report for these meals at the scheduled hours. In exchange for these meals, which will be at no cost to me, I will eat no food or drink any fluids (water excepted) other than that which is provided at the Olin Health Center Cafeteria. I also permit the execution of the tests and measurements as shown in the attached Calendar of Events.

The blood samples will be drawn by personnel in the laboratory of the Olin Health Center. Containers for the collection of urine and feces will be provided by the Food Science Department.

In giving my consent to participate in this study, I agree that all the following statements are true:

(1) I understand that the blood, urine and stool samples are to be used for scientific research. This study has been explained to me and I am aware of any risk involved therein, including the normal risks incident to the withdrawal of blood by needle and syringe.

(2) I have not been coerced in any way to participate in this study, and my consent to participate in it has been given voluntarily. I understand that I am free to discontinue participation in the experiment at any time. If I do so, there will be no recrimination; however, I will not be eligible for the payment which is to be made to the subjects on completion of the study.

(3) I understand that all results of the study will be held in strict confidence and that I will remain completely anonymous in any reports relating to the study.

(4) I understand that, if I so desire, I will be given a summary of the results obtained during this study. Such summary will be available sometime after the completion of the study.

(5) I understand that if I adhere to the schedule of eating three meals each day at the scheduled hours in the Olin Health Center Cafeteria, if I eat or drink (water excepted) nothing other than that available to me through the dining room,if I allow my blood sample to be drawn, and collect urine and stool samples according to the schedule for tests and measurement mentioned above, I will be paid ______ shortly after the completion of the study.

____

Signature of subject

____

Project Leader

____

Date

Appendix C

QUESTIONNAIRE FOR WHEAT DIET AND BLOOD UREA LEVEL STUDY

Name________________________________

Address______________________________

Phone Number(s)______________________

1. Are you on any diet which has been prescribed for health reasons?
   Yes___ No___

2. Are you currently on a weight reducing program?
   Yes___ No___

3. Are you now on any prescribed medication?
   Yes___ No___

4. Have you even been told that you have a kidney problem?
   Yes___ No___

5. Will you be able to eat 3 meals a day in Olin Health Center Cafeteria for 44 days?
   Yes___ No___

6. Is your schedule during Fall Quarter such that you can report to the Olin Health Center Cafeteria for breakfast between 7:00 and 8:00 a.m. (Saturdays and Sundays 7:30 to 8:30 a.m.), for lunch between 11:30 a.m. and 1:00 p.m., and dinner between 5:00 and 6:00 p.m.?
   Yes___ No___

7. Will you, throughout the entire period of study, be able to refrain from strenuous physical activity that might result in sweating?
   Yes___ No___

8. What is your: Age______; Weight______; Height______

9. Are you married?
   Yes___ No___

Appendix D

SCHEDULE FOR MSU BREAD STUDY Fall 1975

Date	Diet	Blood (Fasting)	Urine (24-hr.)	Stool (Complete 24-hr.)
	PERIOD I			
Oct. 13-14	Regular Diet	--[1]	--	--
15	"	X[2]	--	--
16-22	"	--	--	--
23-27	"	--	X	--
28-30	"	--	X	X
31	"	X	X	--
	PERIOD II			
Nov. 1-2	Regular Diet + $NaHCO_3$	--	X	--
3-5	"	--	X	X
	PERIOD III			
Nov. 6	Regular Diet + Bread	X	X	--
7	"	--	X	--
	PERIOD IV			
Nov. 8-9	Bread Diet	--	X	--
10	"	X	X	--
11-13	"	--	X	--
14	"	X	X	X
15-17	"	--	X	X
18	"	X	X	X
19	"	--	X	X
20	"	X	X	X
21-22	"	--	X	X
	PERIOD V			
Nov. 23	Regular Diet	--	--	--
24	"	--	--	--
25	"	X	X	--
26	"	--	--	--
27	"	--	--	--
28	"	--	--	--
29	"	X	X	--

PERIOD VI

Nov.	30	Bread Diet	--	--	--
Dec.	1	"	--	--	--
	2	"	--	--	--
	3	"	X	X	--
	4	"	--	--	--
	5	"	--	--	--
	6	"	X	X	

1. -- No sample collected.

2. X Sample collected.

APPENDIX E

Body weights in pounds of individuals fed (1) wheat gluten liquid diet alone and (2) wheat gluten liquid plus 10 g sodium bicarbonate. Each dietary period lasted 7 days and the weights were recorded at the end of each dietary period.

Subjects	Initial weight	Wheat gluten liquid diet	Wheat gluten diet plus sodium bicarbonate
D.W.	194	194	192
F.B.S.	171	170	169
C.B.	145	145	143
M.A.	154	154	154
Average±S.D.	166±21	166±21	164±21

APPENDIX F

Body weights in pounds of the individuals fed isocaloric and isonitrogenous (1) meat and (2) potato diets respectively. Each dietary period lasted 7 days and weights were recorded on every other day.

	MEAT DIET				POTATO DIET		
Subject/days	1	3	5	7	9	11	13
J.I.	143	142	142	142	142	141	141
D.K.	192	192	191	191	191	190	190
L.D.	146	145	145	146	145	145	145
T.O.	120	130	120	120	120	121	120
S.W.	141	142	142	142	141	140	141
D.W.	196	195	195	195	195	194	194
Average±S.D.	156±30	156±30	155±30	156±30	155±30	155±29	155±29