NITROGEN BALANCE IN COLLEGE WOMEN

THE EFFECT OF COFFEE ON THE NITROGEN BALANCE

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INTRODUCTION

Protein has long been recognized as an essential dietary substance, necessary for the building and repair of body tissue. However, in recent years we have learned even more of the versatility of protein in body processes. In addition to the need of protein for growth and repair of tissue breakdown, protein compounds are now known to serve also as parts of hormones to regulate body processes, enzymes to digest foods, plasma albumin to maintain water balance and blood volume, globulins to resist infection, and hemoglobin to transport oxygen to the tissues.

In times of food shortages, protein deficiency is more likely to occur than that of any other of the important dietary essentials. This is due to the fact that there is a shortage of the foods that furnish protein of high biological value, i.e. the proteins of meat, milk, eggs and cheese. At the present time, where food shortages exist, these foods are scarce and when available expensive. Because of this situation, research concerned with protein metabolism is especially significant today.

Proteins differ from one another on the basis of the number and kind of amino acids of which they are made. Since there are nine (possibly 10) essential amino acids, that cannot be synthesized in the body in adequate amounts from other amino acids, they must be present in the food. These essential amino acids are found in protein of animal origin. Plant protein is good for supplementing other protein,

but alone, it is inadequate for maintenance and growth in the body. With complete protein foods both scarce and expensive, a knowledge of the protein requirement, the factors that affect its utilization, and how the body needs are met are of prime importance.

The protein requirement has been determined from the data obtained from protein (or nitrogen) balance experiments in man. However, due to the nature of the data and the many variable factors involved in the amino acid content of various dietaries, there have been disagreements as to the real requirement, some advocating a high intake, others suggesting a lower intake. Today a compromise of the two extremes is generally advocated. The National Research Council has suggested an adult protein allowance of 60 to 70 gm. per day, from a diet of mixed protein (both animal and plant foods).

In view of the fact that the number of studies to determine the protein requirement of human beings has of necessity been small, this problem was originally undertaken to observe the effect of the variation of caloric intake on protein use by means of the nitrogen balance experiment.

In developing a low protein diet for the above study, coffee was suggested by one of the subjects to make the diet more palatable. This raised the question of whether or not coffee might in some way affect protein utilization or nitrogen excretion. A search was made for data on this point. In reviewing the available litera-

ture it was found that workers between 1853 and 1912 had done some work on the effect of caffeine on the nitrogen in the urine of men and dogs, but their results were contradictory, and in most cases they had used varying and unspecified amounts of caffeine. Since that time this problem has apparently been neglected.

In the hope of obtaining further information concerning the effect of coffee on nitrogen metabolism, this study was planned with the following purposes: To

1. Develop dietary and laboratory technique for the nitrogen balance experiment.
2. Attempt to reach the point of nitrogen equilibrium in subjects on a known protein and caloric intake.
3. Determine the effect, if any, of the ingestion of a known amount of coffee on the total nitrogen, urea and ammonia excretions.

REVIEW OF LITERATURE

EARLY HISTORY OF PROTEIN RESEARCH

A new era of progress, which brought about the glorification of the proteins that remained up to the early part of the present century, was introduced when Francis Magendie (1783-1855) concluded from feeding experiments with livestock that the nitrogen of the tissues is derived from food nitrogen.

G.J. Mulder, a Dutch chemist, gave the name "protein" meaning primary, in 1840, to these substances and stated "It is one of the most complicated substances, is very changeable in composition and is undoubtedly the most important of all known substances in the organic kingdom".

The studies of Bouissingault, in 1851, showed that the protein or nutritive value of a food or feed could be estimated from the nitrogen content alone. This was strengthened by the publications of Liebig who believed that vegetable and animal products contain more or less identical protein substances.

Pettenkofer and Voit, in 1866, found that proteins are not needed for energy, if sufficient carbohydrate and fat are furnished. Voit, in 1872, found that the functions of protein cannot be met without considering the amino acid content of the protein. In 1897, Rubner saw that proteins of varying origin had different biological values. Osborne's and Mendel's experiments on the nutritive value

of proteins "of known character in respect to the relative proportions of their decomposition products" showed that certain amino acids can be synthesized by the growing rodent, while other amino acids cannot and have to be supplied in the food.

These studies on the nutritive value of purified proteins, with similar investigations begun about the same time by McCollum, Funk, Hart, Steenbock, Hopkins and others, led to the discovery of vitamins. This resulted in almost complete abandonment of experiments on the nutritive value of proteins.

The emphasis in protein research since the early twenties has been on the amino acid content of foods and the biological value of each in the body.

In 1935, Rose was able to specify all the amino acids required for normal growth of the albino rat. Rose and his co-workers, in 1942-43, reported that methionine, threonine, leucine, isoleucine, and phenylalanine were indispensable for maintaining nitrogen equilibrium in the human adult. The John Hopkins Hospital also reported that tryptophan and lysine were found indispensable in balance studies on human subjects.

THE COMPOSITION AND USE OF PROTEIN IN THE BODY

Protein is used as a group name for nitrogenous compounds formed by the union of many amino acids, probably linked through abstraction of a molecule of water from an amino group of one and a

carboxyl group of another. Protein is composed of carbon, hydrogen, oxygen, sulfur, nitrogen, and frequently phosphorus and iron. In the body, these elements are combined to form muscle tissues, cellular tissue (nucleoprotein, forming the nuclei of the cells, which breaks down into purins) blood, brain and nerve cells.

The food proteins, after their digestion into amino acids serve several functions. The amino acids carried by the blood to the cells are synthesized into the proteins characteristic of the cells. Each cell selects a certain number of amino acids of the type necessary for the reconstruction of its proteins and protoplasm. The amino acids are used in the manufacture of enzymes, genes, hormones, and other nitrogenous cellular products. From them are made the blood proteins which are indispensable because of their colloidal osmotic pressure and for the maintenance of the acid-base balance. Protein not needed for a specific purpose is deaminated, the amino group split off from the amino acid and the residue used for the production of energy.

THE BIOLOGICAL VALUE OF PROTEINS

The nutritional or biological value of a protein or mixture of proteins depends on the particular kinds of amino acids it contains. In body tissues, if any specific amino acid is entirely lacking or available only in inadequate quantities, the muscle protein cannot be synthesized. The body can transform some amino acids to other kinds

but there are others that cannot be supplied adequately in this way and must be in the food. Proteins containing these essential amino acids are said to have high biological value.

In addition to the amino acid content biological value also depends upon the amount of hydrolysis which occurs in digestion, the level of protein in the dietary, its preparation and the rate at which it passes through the alimentary tract.

Several methods have been used in studying the biological value of proteins, most of them very indirect and with varying results. In 1909 Thomas used the nitrogen balance method which has been used by later workers with some modifications. Mitchel uesd the formula:

$$\text{Biological value} = 100 \times \frac{\text{Body nitrogen spared}}{\text{Food nitrogen absorbed}}$$

Osborne, Mendel, and Ferry (1919) used the growth and maintenance method with the formula:

$$\text{Biological value} = \frac{\text{Gain in weight}}{\text{Intake of protein}}$$

McCollum and Simonds (1929) modified the above procedure by determining the actual protein (N x 6.25) retention which is possible when a given protein is consumed at an arbitrary level of intake.

Mitchell and Beadles (1929-30) devised the paired feeding method, which is especially good for studying the supplemental relationships between proteins and amino acids in which the animals

may be deficient. There is no really good method for studying the nutritive value of proteins, however any of the above can give an idea of the comparative value of different foods.

In the last few years considerable progress has been made in accumulating information about the amino acid make-up of proteins by feeding experiments with animals, by actually isolating individual amino acids from proteins, and by a method of Van Slyke which gives figures for eight fractions of the nitrogen of a protein.

In general, proteins of animal origin have a higher nutritive value than those of any other class. This is understandable, particularly in the case of milk and eggs, since these proteins must provide all the amino acids required by the young animal and developing chick.

THE PROTEIN REQUIREMENTS OF THE BODY

The amount of protein required per day is a matter, concerning which there is considerable difference of opinion. However, according to Mona Spiegel (Wohl 1945) it may be said that there are three levels of protein requirements: (1) the minimal or emergency level, 18-40 gm. daily, barely sufficient for life and health, at least for a restricted time; (2) the average level, 70-80 gm. recommended by the National Research Council and (4) the optimal level 110-140 gm.

Adequate protein must be provided for the wear and tear

which the body sustains and for other needs. When these demands are met, the body is said to be in nitrogen equilibrium and its actual requirement for protein has been met.

Increasing the amount of protein in the food beyond that needed to replace the endogenous protein loss will result in its use for energy. If the diet is already adequate in carbohydrate and fat calories, the excess energy supplied will be stored as body fat, causing an increase in weight. While a high protein diet may throw an extra burden on the digestive and excretory organs and lead to increased intestinal putrifaction, there is little evidence of harmful effects to normal individuals on diets moderately high in proteins. In fact, recent reports by Sako (1943) seem to indicate that "a high protein diet is essential to safeguard against infection".

On a low level of nitrogenous equilibrium the amount of nitrogen in the food may be sufficient to cover all the protein catabolized, but the body may need more energy than it obtains from the ingested food, and to obtain it, body stores of fat are attacked and the body loses weight. If this continues until the fat is depleted, the protein intake may be partly utilized for energy and the nitrogen equilibrium would be lost, resulting in more tissue destruction than regeneration.

Due to the incompleteness of our knowledge, and the fact that vegetable proteins are deficient in certain amino acids, there

may be an advantage in having an abundance rather than a minimum of amino acids available at all times. For this reason a generous allowance of mixed protein is generally recommended for all people, with particular emphasis on protein needs for growth, pregnancy and debilitating diseases.

THE NATURE OF PROTEIN METABOLISM

Digestion

The chemical processes of digestion, by a series of specific enzymes under optimal conditions, accomplish the breakdown of the complex protein molecules into simpler, smaller, more soluble, and more diffusible molecules. This makes possible the absorption of amino acids through the intestinal mucosa and provides material for the synthesis of proteins characteristic of the body rather than the food source.

Absorption

"Folin and Denis (1912) clearly demonstrated that, as amino acids are absorbed from the intestine their concentration first rises in the portal blood, then in the liver, and as the blood passes from the liver into the systemic circulation, the latter shows a high tide of amino acids. The high level in the blood soon falls, while that of the muscles and the organs rises, showing that these tissues remove amino acids from the blood. Simultaneously with the flooding

of the liver, systemic blood, and tissues with amino acids, the formation of urea begins, and continues until the blood and tissue content of these acids returns to the fasting level; after absorption from the intestines is completed. Van Slyke and Meyer (1912) confirmed Folin and Denis' findings in every detail, using Van Slyke's direct method for estimating amino acids" (Wohl 1945).

Utilization

It is not known exactly how the cell protoplasm takes the necessary amino acids from the tissue fluid and synthesizes them into its own specific protein. According to the theory of Bergmann and Niemann 1937, the building of amino acids into proteins is a function of certain enzymes always found in the cells of the body, which being capable of synthesizing must also be able to split up the compounds they form. The enzymes facilitate the synthesis of these tissue proteins or their destruction depending upon the point of equilibrium of the substrate. When the supply of amino acid is sufficient, the enzymes build up tissue protein; when the supply of amino acid is decreased, the proteins are decomposed.

Excretion

The destruction of protein in the body gives rise to two classes of waste products, the nitrogenous and the non-nitrogenous. The non-nitrogenous include carbon dioxide and water, which are also produced by the catabolism of fats and carbohydrates.

The nitrogenous products are peculiar to protein catabolism, since the proteins are the only foods that contain nitrogen. The most important of these products are urea, ammonia salts, uric acid and creatinine. In addition to these, the proteins give rise to waste products containing nitrogen, sulfur, and phosphorus, which are excreted exclusively by the kidneys.

It is generally known that a certain amount of the amino acids carried by the portal vein from the intestines to the liver is deaminated and the nitrogenous part converted into urea by the liver. In the wear and tear which the proteins sustain during cellular activity, amino acids are also deaminated; the resulting ammonium carbonate is carried to the liver to be transformed into urea, which is the principal end product of protein metabolism in the body. The ammonium salts excreted in the urine are chiefly those of the inorganic acids, for the organic ammonium salts are largely transformed into urea.

Uric acid is the final waste product of nucleoproteins and nucleins. The uric acid formed from ingested food is known as "exogenous" and that from the nuclear material of the tissues as "endogenous" uric acid.

Creatinine, like uric acid, is of both exogenous and endogenous origin. The endogenoue creatinine is considered to be fairly constant and is taken as an index of the real tissue wear

and tear.

NITROGENOUS EQUILIBRIUM IN THE BODY

When the nitrogen intake and excretions are the same, the body is said to be in nitrogen or protein balance. If the intake is greater than the excretion, nitrogen is being retained or stored in the body, and the body is said to be in positive balance. When the nitrogen excretions exceed the intake the body is in negative balance, and the needs of the body for protein are not being adequately supplied.

Levels of Equilibrium

Nitrogenous equilibrium can be established at various levels, either with a high or low protein diet, for the body tends to adjust itself to the level of protein intake, provided the essential amino acids are supplied. The time needed for the adjustment depends upon the difference between the new level and the protein intake to which the body has been accustomed. With a very high level of protein, the excess will be deaminated and used for energy, and if there is sufficient carbohydrate and fat in the diet, a gain in weight will result. On the moderately low levels of protein intake, carbohydrates and fats furnish most of the energy, and the protein if made up of the proper amino acids will be adequate for maintenance and health.

Chittenden (1913) made extensive experiments showing that

balance and health could be maintained in professional men, soldiers and athletes on a moderately low level of protein (44-53 gm. protein for a 70 kg. man, on the average). Sherman made a study in 1920 of 109 experiments in 25 different investigations and found that an average of 44.4 gm. per 70 kg. man could be taken as an apparent requirement. However, Sherman, Voit (1860), Atwater (1903), Christianson (1934), Susskind (1934) Hinhede (1934) and others favor a higher protein allowance than this for optimal maintenance, growth and health.

Nitrogen Minimum Excretion

To determine the effect of a protein free diet on the nitrogen excretion, and the nitrogen minimum, Deuel (1928) lived for 54 days on a diet nearly free from protein, (o.24, 0.32-o.51 gm nitrogen). He found that there was an immediate and progressive decrease in the total nitrogen excretion. This was very rapid at first but declined more slowly after about 9 days. His nearest approach to nitrogen minimum, or level of tissue wear and tear, was 1.79 and 1.75 gm. or 24.7 and 24.1 mg. per kg. of body weight. Thomas (1910) obtained a 26.6 mg. per kg. level and Smith (1926) 0.0242 gm. per kg. It has been pointed out, however, that there are larger amounts of nitrogen in the feces on the high carbohydrate, protein-free diets. This occurs because there is a low urea output in the urine under these conditions. Basu and Basak (1939) found that in two Indian subjects

the minimum nitrogen excretions were 1.499 and 2.302 gm. in the urine and 0.946 gm. and 1.133 gm. in the feces, a total of 0.05-0.07 gm. per kg.

Even on a protein-free diet, as shown in the above, the body tends toward balance. As the time is extended, the negative balance decreases very slowly but surely. If this continues long enough, death would result when the nitrogen excretion reached balance if not before.

Factors Affecting Equilibrium

The levels of carbohydrate, fat and protein are all factors in nitrogenous equilibrium in the body. Lusk (1890) showed that a sudden withdrawal of carbohydrate from the diet would increase the nitrogen excretion materially, making a greater negative balance. This showed that carbohydrate acts as a protein sparer. From the experiments of Kayser (1893) and Tallquist (1902), it seems that if carbohydrate is almost entirely replaced by equal fat calories, it is unfavorable to nitrogen balance. When fat calories make up only one-half of the total calories, the difference in protein sparing action is but slight.

Elman, Davey, and Kiyasu (1945) found in experimenting with dogs on a diet of 25-50 calories per kg. of body weight, that the dogs on a diet of 80% protein and 20% carbohydrate were in marked positive balance on both caloric levels. With the 80% carbohydrate and 20% protein diet, one dog was in positive balance on the 50 cal-

ories per kg. level. This would suggest that balance is more easily obtained on a high level of protein than on a low level and that better physiological results may be obtained by increasing protein in the diet and decreasing the carbohydrates, rather than the reverse as some people have advocated.

The type of protein foods used will also have some effect on the nitrogenous balance. A number of experiments using different sources and various mixtures of proteins have been made and equilibrium has been obtained at comparatively low levels, even with proteins that are not of the highest nutritional value. Rose and Cooper (1917) showed that equilibrium could be reached with potato protein at a level of 0.5 gm. per kg. of body weight. Sherman and Winters (1918) showed similar results using nine-tenths of the protein from corn and one tenth from milk. In general, however, it may be stated that the more closely the protein of the dietary mixture approaches the exact composition of the body proteins, the less protein will have to be fed to balance the needs of the body.

THE PHYSIOLOGICAL EFFECTS OF COFFEE

Constituents of Coffee Beverage

The important water soluble products in coffee are tannin, caffeol, caramelized carbohydrates, and carbon dioxide.

Ukers (1922) believes the tannin substances to be misnamed and harmless. Caffeol gives coffee its characteristic taste, is very

volatile and is present is such small quantities that it is harmless. Caffeine is the alkaloid that may act as a stimulant in coffee. Mac Leod and Nason (1937) state that the concentration of caffeine in the average cup of coffee is from 1.5 to 1.75 grains, about 80% of it being extracted in the first 2 minutes in coffee making by any of the usual methods.

Effect of Coffee and of Caffeine on the Body

"Coffee, used in moderation, is a valuable stimulant increasing personal efficiency in mental and physical labor. The action in the alimentary tract is that of an adjuvant food, aiding digestion, favoring increased flow of the digestive juice and promoting intestinal peristalsis. It reacts on the kidneys as a diuretic, and increases the excretion of uric acid. Coffee has been indicated as a specific for various diseases, its action being the raising and sustaining of low vitalities. Coffee and caffeine are physiologically antagonistic to the common narcotics, nicotine, morphine, opium, and alcohol and are frequently used as antidotes for these poisons". (Ukers 1922)

Ukers quotes from the British Pharmaceutical Codex that caffeine exerts three important actions in the human body. The first of these is on the central nervous system, producing a condition of wakefulness and increased mental activity when taken in moderation. The second action is on muscles including the cardiac, facilitating the performance of all kinds of physical work, actually increasing

the total work which can be obtained from muscle, accelerating the pulse and slightly raising blood pressure. The third action is on the kidney, producing an initial vaso-constriction by exciting the medulla, which at first retards the flow of urine but shortly after exerts a diuretic effect.

The question of the influence of caffeine upon tissue changes and the consequent nitrogenous elimination has not been distinctly answered, although Ukers states that the most probable conclusion is that the action of caffeine upon urea elimination and upon the general nutrition is not direct or pronounced.

In the Bernheim,F. and Bernheim, M report (1945) on the effect of caffeine on the metabolism of liver and kidney slices in vitro, caffeine was found to interfere in both tissues, with the utilization of added ammonium salts, and in liver it inhibited the formation of urea. It was observed however, that if sufficient amounts of ornithine or glutamine are present the inhibition can be overcome.

Earlier workers (Lehmann, 1853, Robuteau, 1870, Roux, 1874, Farr and Walker, 1912) investigated the nitrogen excretion in the urine after varying and in most cases unspecified amounts of caffeine had been administered. Their results were contradictory. Since that time the problem has apparently been neglected.

As Bernheim and Bernheim point out "since the caffein inhibition of urea formation from ammonia is so definite in vitro,

it will be of interest to determine whether it also occurs in vivo. That some of the earlier workers found a low urea excretion after caffeine and others did not, indicated that certain conditions may be necessary before the effect is demonstrable in the whole animal. The fact that caffeine can interfere with the nitrogen metabolism in the liver and kidney may eventually explain its effects on the basal metabolic rate, and on the production of prothrombin."

Haldi, Bachman, Ensor and Wynn (1944) have shown the rate of absorption and the action of caffeine in coffee and tea is the same as that of the pure alkaloid in solution.

In the light of these findings this study was planned to find the level of nitrogen equilibrium in two subjects on a known protein intake and to determine the effect, if any, of coffee on the total nitrogen balance and on the urea amd ammonia excretions of these subject.

EXPERIMENTAL PROCEDURES

BIOLOGICAL METHOD

Experimental Subjects

The two women who served as experimental subjects for this study were selected from a group of four who were on a previous experimental period for nitrogen balance on normal and low protein diets. Since the amount of analytical work involved is great, it was found that fewer subjects should be used.

The subjects were living in the dormitories at the Virginia Polytechnic Institute during the winter and spring of 1946 and each was doing graduate research and attending classes at the time.

The subjects maintained average good health throughout the experimental period, with the exception of the first day of the test period when both were ill, probably from some toxic substance contracted in the laboratory. Subject II maintained her normal weight of 131 pounds throughout the experiment. Subject I, weighing 123½ pounds in the beginning, lost steadily until the last supplemental period, at which time she weighed 121.5 pounds. An effort was made to increase calories to prevent the loss, but in spite of this the weight loss persisted. These additional calories were chiefly from fat due to the sugar shortage.

Basal Diet

This basal diet was preceded by a two day period in which

all animal protein foods were excluded from the diet.

Period I. This basal diet was designed to bring the subjects into negative balance, supplying a minimum of poor quality protein foods with an adequate supply of all other nutrients. The diet was originally planned to furnish 2000 calories but due to difficulties presented by the local food situation, actually contained only 1726 calories. Tables 1 and 2 give the actual food intake for each subject, and tables 3 and 4 give their percentage composition. This basic diet, when tested with a Kjeldahl analysis was found to contain 1.07 grams of nitrogen representing 6.69 grams of protein. The diet became very monotonous but was well tolerated by both subjects.

Supplementary Feedings

Period II. This first supplement to the basic diet was based on the theoretical amount of protein needed to bring the negative balance of Period I up to the point of nitrogen equilibrium. This supplement consisted of 200 grams of whole milk (1.14 gm. nitrogen) and 49 gm. of whole egg (1.02 gm. nitrogen) given in addition to the the basal diet of Period I. Increased sugar and fat were given to Subject I in an attempt to check weight loss. Slightly less calories of the Basic diet was supplied for Subject II, although the new caloric total was slightly larger than the previous total. The total nitrogen intake for both subjects in this period was 3.23 grams of nitrogen or 20.34 grams of protein.

Table 1

Experimental Diet - Subject I

Food	Measure	Protein Grams	Calories	Reaction Acid	Reaction Base
Orange juice	190 cc.	—	75.0		11.2
Lemon	60 cc.	—	17.4		2.4
Distilled water	690 cc.	—	—		
Applesauce	115 gm.	0.2	52.0		2.8
Grape jelly	60 gm.	—	180.0		
Oleomargarine	28 gm.	—	204.0	Neutral	
French dressing	45 gm.	0.3	192.0	Neutral	
Carrots	100 gm.	1.0	36.0		14.0
String beans	100 gm.	1.0	18.0		
Lettuce	100 gm.	1.0	20.0		7.4
Cornstarch Cookies	120 gm.	1.1	444.0	6.89?	
Sugar	7-9 Tb.	—	420.0	Neutral	
Fondant	1 Tb.	—	66.4	Neutral	
Minerals		—	—		
Vitamins		—	—		
Distilled water	750 cc.	—	—		
Diet I total		6.69[1]	1726	6.89	37.8
Diet I (modified)		6.69	1882	6.89	37.8
Milk, whole[2]	200 gm.	7.27	138		3.6
Eggs, whole	49 gm.	6.38	70	5.67	
Diet II total		20.34	2090	12.56	41.4
Diet I (modified)		6.69	1882	6.89	37.8
Milk, whole	256 gm.	9.31	177		4.3
Egg, whole	65 gm.	8.44	93	7.5	
Diet III total		24.44	2152	16.39	42.1
Diet III		24.44	2152	16.39	42.1
Coffee	968 cc.	1.50	6		
Diet IV total		25.94	2158	16.39	42.1

[1] Determined by Kjeldahl

[2] 1 gm. N = 6.38 gm. of milk protein or 6.25 gm. of other protein

Table 2

Experimental Diet - Subject II

Food	Measure	Protein Grams	Calories	Reaction Acid	Reaction Base
Orange juice	190 cc.	—	75.0		11.2
Lemon	60 cc.	—	17.4		2.4
Distilled water	690 cc.	—	—		
Applesauce	115 gm.	0.2	52.0		2.8
Grape jelly	60 gm.	—	180.0		
Oleomargarine	28 gm.	—	204.0	Neutral	
French dressing	45 gm.	0.3	192.0	Neutral	
Carrots	100 gm.	1.0	36.0		14.0
String beans	100 gm.	1.0	18.0		
Lettuce	100 gm.	1.0	20.0		7.4
Cornstarch cookies	120 gm.	1.1	444.0	6.89?	
Sugar	7-9 Tb,	—	420.0	Neutral	
Fondant	1 Tb.	—	66.4	Neutral	
Minerals		—	—		
Vitamins		—	—		
Distilled water	750 cc.	—	—		
Diet I total		6.69[1]	1726	6.89	37.8
Diet I (modified)		6.69	1617	6.89	37.8
Milk, whole [2]	200 gm.	7.27	138		3.6
Eggs, whole	49 gm.	6.38	70	5.67	
Diet II total		20.34	1825	12.56	41.4
Diet I (modified)		6.69	1617	6.98	37.8
Milk, whole	228 gm.	8.29	158		4.1
Egg, whole	61 gm.	7.94	87	7.10	
Diet III total		22.92	1862	13.99	41.9
Diet III		22.92	1862	13.99	41.9
Coffee	968 cc.	1.50	6		
Diet IV total		24.42	1868	13.99	41.9

[1] Determined by Kjeldahl

[2] 1 gm. N = 6.38 gm. of milk protein or 6.25 gm. of other protein

Period III. Additional supplements were needed to obtain balance, so this second supplementary period was introduced. In this period Subject I received 266 grams of milk (1.46 gm. nitrogen) and 65 gm. of egg (1.35 gm. nitrogen) making a total intake of 3.88 gm. of nitrogen or 24.44 grams of protein, calculated to the amount needed to reach balance. Subject II received 228 gm. of milk (1.3 gm nitrogen) and 61 gm. egg (1.27 gm. nitrogen) in addition to the basic diet, making a total of 3.64 grams nitrogen or 23 gm. protein ingested. Subject II reached balance on this diet but Subject I did not.

Period IV. This third supplement consisted of 968 cc of coffee (0.24 gm. nitrogen) given in place of the distilled water for drinking in the previous periods. All other foods remained the same as in Period III for both subjects. The purpose of this period was to observe the effect of coffee on nitrogen balance and on the level of urea and ammonia in the urinary nitrogen excretion.

The coffee in this diet is estimated to contain about 12 grains or .77 gm. of caffeine.

CHEMICAL METHOD

Collection of Samples

Urine was collected for 24 hour periods beginning at 8 a.m. in bottles containing 15 milliliters of glacial acetic acid as a

preservative. The specific gravity of the urine was determined and recorded. The original volume was measured and the urine diluted to one liter or to the nearest hundred milliliters if the volume was greater than one liter. This increased volume was recorded and used in all calculations. The sample was shaken thoroughly and a portion poured into a amaller container from which samples for analyses were taken.

Tests were made at intervals on the urine for glucose and albumin, using the Benedict and Heller's Ring tests.

Fecal samples were collected in glass casseroles for 24 hour periods beginning at 8 a.m. The samples were covered with 25 milliliters of glacial acetic acid. They were homogenized by the use of a policeman, distilled water and a strainer when necessary. Transfers were made with minimum loss. Pools were made of 1 to 3 day's samples. The pools were carefully weighed, and weighed samples for analysis were taken directly from the pool. Markers were not used as they were not found to be particularly helpful in a previous period.

Determination of Total Nitrogen Excretion

The Kjeldahl method was used for the determination of total nitrogen. In this method the various nitrogenous substances of the sample are converted into ammonium sulfate by boiling with sulfuric acid. The ammonium sulfate is then decomposed by

means of strong sodium hydroxide and the liberated ammonia collected in an acid of known strength. By titrating this partly neutralized acid solution with an alkali of known strength the nitrogen content of the sample is computed.

Accurately measured 5 milliliter urine samples and 10 gm. fecal samples were digested with 20 milliliters of concentrated sulfuric acid, distilled with 80-90 milliliters of strong sodium hydroxide and the ammonia liberated was collected in .5 normal hydrochloric acid and titrated with .1 normal sodium hydroxide.

In calculating the nitrogen in the sample, the milliliters of .1 normal sodium hydroxide used in titrating were subtracted from the number of milliliters of .1 normal hydrochloric acid used (5x number of milliliters of .5 normal hydrochloric acid) which gave the number of milliliters neutralized by the ammonia from the sample. Since 1 milliliter of .1 normal hydrochloric acid contains .0014 gm. of nitrogen the number of milliliters neutralized was multiplied by .0014 which gave the amount of nitrogen in the sample. This figure was divided by the number of milliliters in the sample to get the number in 1 milliliter from which any desired calculation was made.

All samples were run in duplicate and checked within 1 milliliter of sodium hydroxide in titrating.

Determination of Creatinine in the Urine

In this study, Folin's Microchemical Modification (in Hawk

and Bergeim's Physiological Chemistry) was used in the determination of creatinine. This method is based on the property of creatinine to develop a certain definite color in the presence of picric acid in alkaline solution, a reaction due to the formation of a red tautomer of creatinine picrate.

One milliliter of the standard creatinine solution (1 milligram per milliliter) was measured into a 100 volumetric flask and 1-2 milliliters of urine in another. To each was added 20 milliliters of saturated picric acid and 1.5 milliliters of 10% solution of sodium hydroxide by burette. The flasks were rotated for 30 seconds, and allowed to stand for exactly 10 minutes then were diluted to 100 milliliters, mixed, and compared at once ina colorimeter, set at 0 with a blank reagent solution. The creatinine content was determined by the formula:

$$\frac{\text{Reading of standard}}{\text{Reading of unknown}} = \text{milligrams of creatinine in sample used}$$

Samples were run in duplicate and a new blank and standard made for each series of tests.

Determination of Urea and Ammonia

The method of Van Slyke and Cullen was used for the determination of urea and ammonia. In this method, the enzyme urease, at ordinary temperatures, transforms urea, quickly and completely into ammonium carbonate. The ammonia of the urine is set free by the addi-

tion of an alkali and this ammonia is then carried over by an air current into a tube containing a measured amount of standard acid. The excess acid is then titrated. The method as described in Hawk and Bergeim does not stress pH and temperature but it has been found necessary in some cases to adjust these for proper enzyme action.

A series of 9 test tubes was set up so that tests for total ammonia and urea nitrogen and ammonia nitrogen alone would be made on urine simultaneously and in duplicate, using the same air current, time etc. for aeration. The tubes were lettered as A or B with each A tube joined to a corresponding B tube. The A tubes contained the sample for analysis, the B tubes the acid for collecting the ammonia.

Into tubes A and A_1 (see Figure 1. page 29) were placed 5 milliliters of fresh diluted urine (5 milliliters urine to 50 of distilled water) and 2 drops of caprylic alcohol. Into tubes A_2 and A_3 , 5 milliliters of fresh undiluted urine were pipetted and 2 drops of caprylic alcohol were added. Into all the B tubes were placed 25 milliliters of 0.02 normal hydrochloric acid, 2 drops caprylic alcohol and 5 drops of methyl red indicator. (A sample of dilute urine was tested for pH with phenophthalene, using just enough dilute sodium hydroxide solution to prevent the change of color). Sodium hydroxide solution was dropped into tubes A and A_1 as indicated by the pH check on the sample tested; 2 milliliters of urease solution and three milliliters of buffer solution were added and the tubes

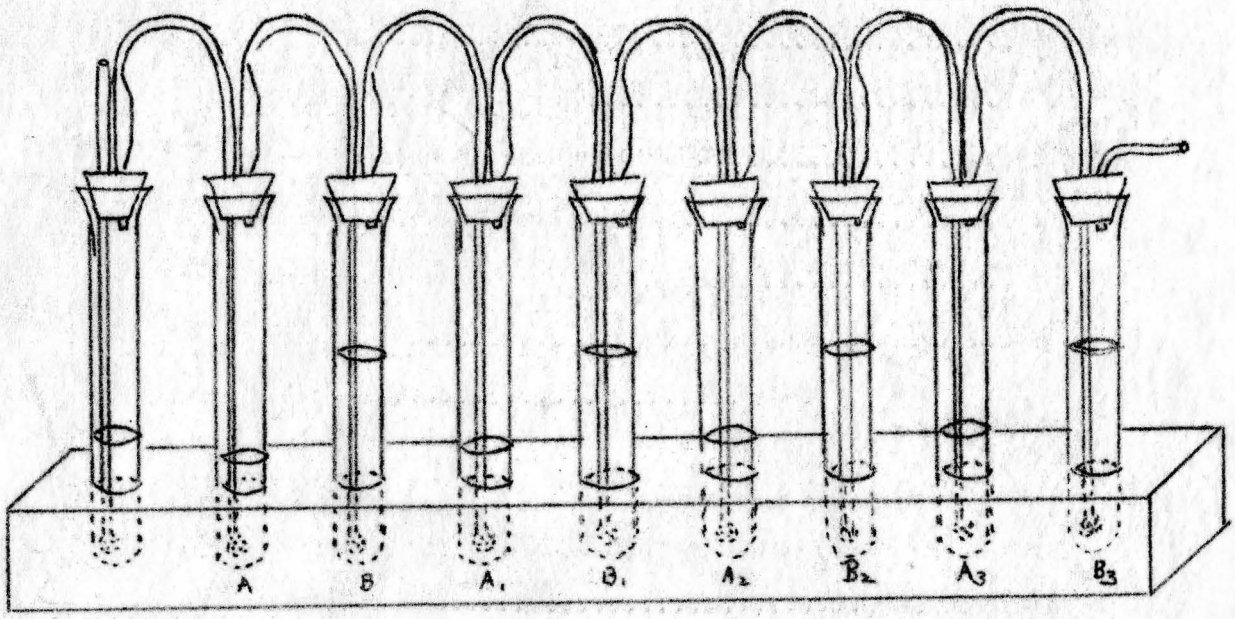


Figure 1. The Ammonia-Urea Aeration Apparatus

closed and placed in beakers of water just under 60 C. and allowed to stand for 30 minutes. The series was then connected with the air current and allowed to aerate for about 3-4 minutes. Five milliliters of saturated potassium carborate was then introduced into all A tubes and the series again aerated for 30 minutes, to carry the ammonia in the A tubes into the B tubes. The excess acid in the B tubes was then titrated with 0.02 normal sodium hydroxide solution.

When checks were obtained for tubes B and B_1 the milliliters of 0.02 normal acid neutralized by the ammonia from the sample is multiplied by 0.056 to give the number of gm. of urea plus amminos in 100 milliliters of the urine. For tubes B_2 and B_3 the number of milliliters of 0.02 normal acid neutralized is multiplied by the fac-

tor 0.0056 to give the number of gm. of ammonia nitrogen in 100 milliliters of urine. By subtracting the figure for ammonia from the figure for ammonia plus urea the figure for urea is found. The total output of each may be calculated from these figures.

RESULTS AND CONCLUSIONS

DIETARY PERIODS AND NITROGEN BALANCE

Four feeding periods were introduced to observe the point of nitrogen balance with milk and egg protein, and to see the effect, if any of coffee on the nitrogen excretion.

Period I. The diet of this period contained 1726 calories for both subjects and was approximately 65% carbohydrate, 33% fat and 1.5% poor quality protein. This relatively high proportion of fat was used because of the shortage of sugar, and the high caloric yield of fat. The caloric needs were adequately met for Subject II but insufficient for Subject I, who lost one pound of weight during this four day period.

When the two subjects began the low protein diet (1.07 gm. nitrogen) a large negative balance was observed. This may be seen in tables 3 and 4. Both the total nitrogen excretion and the negative balance decreased progressively on each of the four days of the period. Deuel (1928) observed that on a non-protein diet this decrease is very rapid at first, but after 6 to 8 days becomes more gradual and irregular, indicating two types of storage protein, one more quickly utilized than the other. It was observed that Subject II excreted more nitrogen each day than Subject I.

The high nitrogen excretion and creatinine output on the first day of the period (see Tables 5 and 6 and Graph I) was due largely to some toxic substance which caused a temporary illness

in both subjects. The average excretion of nitrogen in the feces during this period was .81 gm. and .97 gm. representing about 20% of the average total nitrogen excretion for each subject. This is higher than is usually found on an average diet.

Period II. The diet for this period was calculated, using tables to approach the nitrogen requirement to bring about balance. Additional calories were given Subject I to check weight loss. For both subjects 200 gm. of milk and 48 gm. of egg were added to the diet of period I. Subject I was now receiving about 2090 calories consisting of about 57% carbohydrates, 39% fat and 3.9% protein. Subject II received 1825 calories, 60% carbohydrate, 35% fat and 4.4% protein. This represented a total addition of 35 carbohydrate gm. and 24.4 gm fat for Subject I and 11 gm. carbohydrate and 7.9 gm. fat for Subject II.

Subject I continued to lose weight on this diet, her total weight loss at the end of this period was 3 pounds. Subject I had an average negative balance for the period of -.77 gm. of nitrogen (4.8 gm. protein). On the third day of the period she had a -.08 balance but on the last day it was -1.12 which shows that the adjustment is very irregular. This poor adjustment may be partly due to the extra activities and long hours spent in working on this experiment.

Subject II showed a more regular balance, ranging from +24 to -.20 on the last day. Her average balance for the period was -.37 gm. of nitrogen (2.3 gm. protein). Both subjects were beginning to

Table 3

Nitrogen Balance for Subject I

Intake					Output			Nitrogen Balance
Total Calories	Carbohydrates (%)	Fat (%)	Protein (%)	Food N. (gm.)	Urine N (gm.)	Feces N (gm.)	Total N excretion	
1726	65.2	33.3	1.5	1.07	3.50	.93	4.43	-3.40
1726	65.2	33.3	1.5	1.07	3.48	.93	4.41	-3.34
1726	65.2	33.3	1.5	1.07	2.97	.68	3.65	-2.58
1726	65.2	33.3	1.5	1.07	2.66	.68	3.34	-2.27
2090	57.3	38.8	3.9	3.23	2.77	1.06	3.83	- .60
2090	57.3	38.8	3.9	3.23	2.91	1.77	4.68	-1.45
2090	57.3	38.8	3.9	3.23	2.77	.54	3.31	- .08
2090	57.3	38.8	3.9	3.23	3.33	.54	3.87	- .64
2090	57.3	38.8	3.9	3.23	2.77	1.58	4.35	-1.12
2152	56.2	39.3	4.5	3.88	-	-	-	-
2152	56.2	39.3	4.5	3.88	3.19			
2152	56.2	39.3	4.5	3.88	3.21	.98	4.19	- .31
2152	56.2	39.3	4.5	3.88	3.57	.98	4.55	- .67
2158	56.0	39.2	4.8	4.12	-	-	-	-
2158	56.0	39.2	4.8	4.12	3.52	1.40	4.92	- .80
2158	56.0	39.2	4.8	4.12	3.47	.81	4.28	- .16
2158	56.0	39.2	4.8	4.12	3.67	.81	4.48	- .36

pproach balance but neither could be said to have reached balance in this period.

Period III. In again attempting to reach balance, it was estimated that about 4.8 gm. of protein would be needed for Subject I and about 2.3 gm. for Subject II. Subject I was given a diet of 2153 calories, 56% carbohydrate, 39% fat and 4.5% protein. The caloric

Table 4

Nitrogen Balance for Subject II

Intake					Output			Nitrogen Balance
Total Calories	Carbohydrates	Fat (%)	Protein (%)	Food N (gm.)	Urine N (gm.)	Feces N (gm.)	Total N Excretion	
1726	65.2	33.3	1.5	1.07	5.67	1.15	6.82	-5.75
1726	65.2	33.3	1.5	1.07	3.53	1.15	4.67	-3.60
1726	65.2	33.3	1.5	1.07	3.00	.80	3.80	-2.73
1726	65.2	33.3	1.5	1.07	2.80	.80	3.60	-2.53
1825	60.6	35.0	4.4	3.23	2.99	---	2.99	- .24
1825	60.6	35.0	4.4	3.23	2.59	.98	3.57	- .34
1825	60.6	35.0	4.4	3.23	3.25	.81	4.06	- .83
1825	60.6	35.0	4.4	3.23	2.79	.81	3.60	- .37
1825	60.6	35.0	4.4	3.23	2.74	.69	3.43	- .20
1862	59.7	35.4	4.9	3.64	---	---	---	---
1862	59.7	35.4	4.9	3.64	3.24	.75	3.99	- .35
1862	59.7	35.4	4.9	3.64	3.02	1.03	4.05	- .41
1862	59.7	35.4	4.9	3.64	2.35	1.02	3.37	- .27
1868	59.5	35.3	5.2	3.88	---	---	---	---
1868	59.5	35.3	5.2	3.88	2.67	.98	3.65	- .23
1868	59.5	35.3	5.2	3.88	3.23	.50	3.73	- .15
1868	59.5	35.3	5.2	3.88	3.42	.50	3.92	- .04

intake was again inadequate, for she lost 2 pounds in this four day period. Subject II was given a diet of 1862 calories, 60% carbohydrate, 35% fat and 4.9% protein.

Subject I had an average negative balance of about -.49 (3 gm. protein). The lowest daily balance was -.31. Subject II had an average balance of -.16, the last day having a positive balance of

+.27. This dietary is thought to be very near balance for Subject II, but the diet for Subject I is thought to be about 3 protein gm. short.

Period IV. This period was planned to observe the effect of the ingestion of 968 milliliters of coffee given in addition to the intake of each subject in Period III. The coffee was made in a drip-olator, using 5 Tb. of coffee to 4 cups of water. It is estimated that 968 milliliters of the coffee contained about 12 grains or 0.77 gm. of caffeine. Adding the coffee nitrogen , 0.24 gm. as determined by the kjeldahl analysis, to the figures found for the diet in Period III, the intake, excretions, and balance were figured as before.

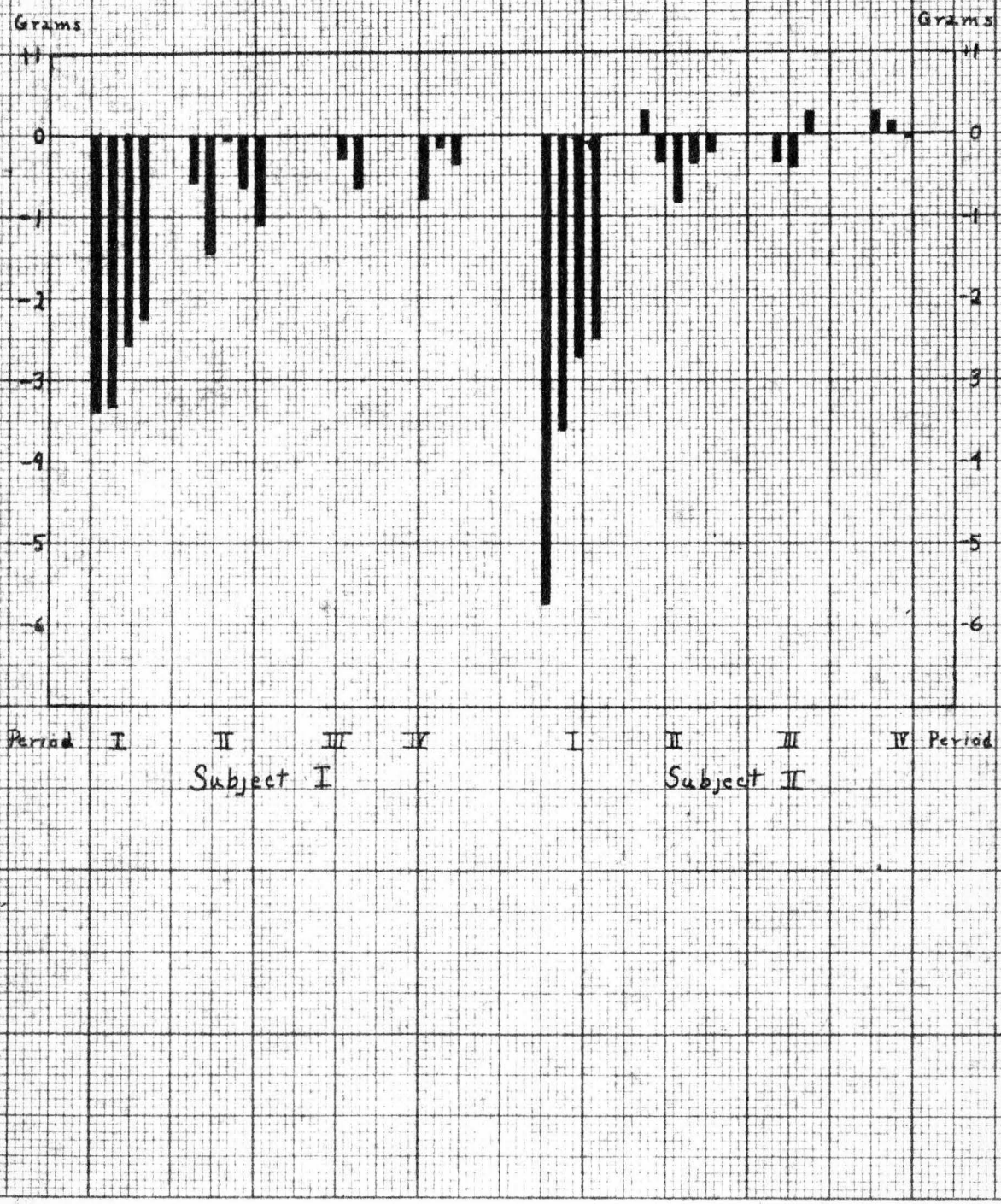
In this coffee period, Subject I did not reach balance, how-the weight loss was checked. She had an average balance of -.44 which is about the same as in the previous period. The nearest approach to balance was made on the third day when the balance was -.16. It would seem that coffee had no effect on nitrogen balance for Subject I.

Subject II was definitely in balance during this period. There was an average + .19 balance on the second and third day and the balance was -.04 or true balance on the last day. This may be interpreted as the tendency of the body to adjust its nitrogen output with its intake when the dietary change is not too great and adequate time is allowed. It would seem that coffee had no effect on balance in Subject II.

From the above findings, it would appear that coffee does not affect the amount of total nitrogen excretion or nitrogenous equilibrium.

GRAPH I

Nitrogen Balance For Subjects I and II on Low Protein Diets



brium.

Due to the fact that carbohydrate has been found to be a better protein sparer than fat, a higher percentage of carbohydrate might have helped to make Subject I go into balance on a lower intake of protein. It will be noted in Table 3 that the percentage of carbohydrate in the diet was decreased 9% while that of Subject II was was decreased only 6%; the fat was increased 6% for Subject I and only 2% of the total calories for Subject II. It is believed that a higher carbohydrate and lower fat intake might have been more favorable to balance for Subject I.

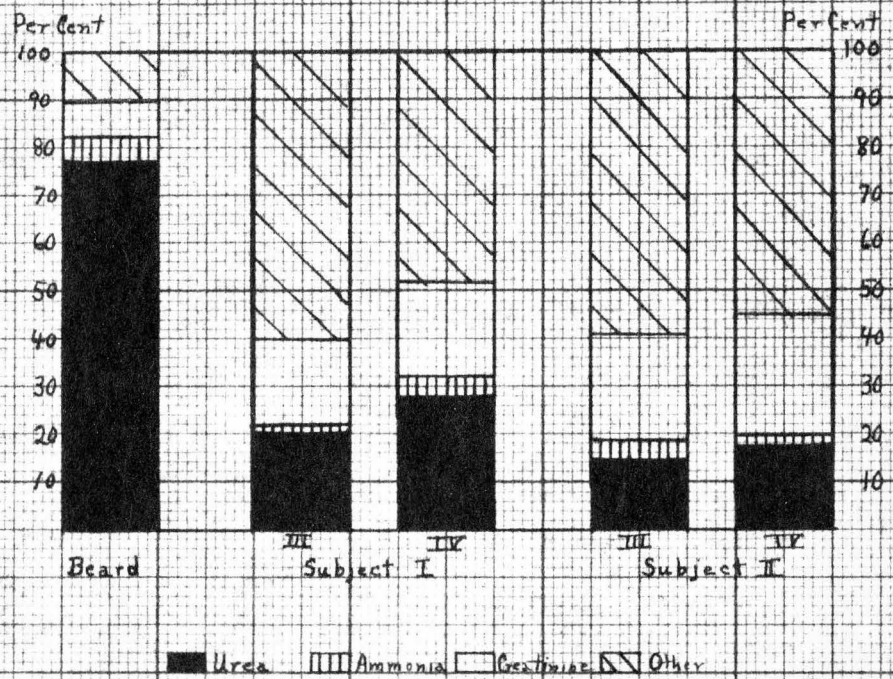
DISTRIBUTION OF URINARY NITROGEN

A comparison for the subjects in this experiment with an average of the partition products found on a low protein diet by Beard is found in Tables 7 and 8. Beard's average nitrogen excretion represents about 50 gm. of protein. This would be considered a low level of protein in the diet but is at least twice that of the level used in Periods III and IV in this study. In comparing Period III of Subjects I and II respectively, with Beard's figures, urea was about 89 and 93% lower, ammonia 66 and 75% lower, and the creatinine output about the same. This would indicate that the values for urea and ammonia in this study are extremely low. Graph 2 gives a good picture of these comparisons.

Folin (1905) found that a decrease in the total nitrogen

GRAPH 2

Comparison of Average Distribution of Urinary Nitrogen for Subjects I and II with the Averages of Beard



excretion was always accompanied by a decrease in the percentage of the total nitrogen excreted as urea, and that after regulating the diet of a normal person to cause the excretion of total nitrogen to be reduced to 3-4 gm. in 24 hours, only about 60% of this nitrogen appeared in the urine as urea. The highest percentage for urea obtained in this study was 40.4%, the lowest was 2.2%. Hawk and Bergeim (1937) state that the average output of ammonia in the urine is about 0.7 gm. ammounting to 2.5 - 4.5% of the total nitrogen excretion. Folin showed that a pronounced decrease in the extent of protein metabolism, as measured by total nitrogen in the urine, is frequently accompanied by a decreased elimination of ammonia. He also found that a decided decrease in the total nitrogen excretion is always accompanied by a relative increase in the ammonia-nitrogen, provided the food does not yield an alkaline ash. All of the diets in this study yield an alkaline ash as shown in Tables 1 and 2.

The lowest ammonia value found in this study was .3% and the highest 5.2%. The period averages however, ranged from 2.2% to 4.1%.

In this study, the figures for urea would seem too low to be plausible. In working with a protein-free diet, Deuel found his lowest urea excretion was 0.75 gm. in 24 hours when the total nitrogen excretion was 1.75 gm. The urea represented 43% of the total

nitrogen excretion.

In Tables 5 and 6 and in Graph 2, the comparative results in Periods III and IV of this study for each subject may be seen. The average ammonia increase for Subject I was 1.6% and 8% for urea. Subject II showed a 1.4% decrease in ammonia and an average 3% increase in average urea excretion during the coffee period.

Table 5

Food Intake and Nitrogen Excretions for Subject I
in the Four Experimental Periods

	Period I				Period II				
	May 7	May 8	May 9	May 10	May 11	May 12	May 13	May 14	May 15
Weight		127.5	127	126.5	126	125.5	125	124.5	124
Intake: Total Calories	1726	1726	1726	1725	2090	2090	2090	2090	2090
Carbohydrate (gm)	281	281	281	281	316	316	316	316	316
Fat (gm)	64	64	64	64	90.4	90.4	90.4	90.4	90.4
Protein (gm)	6.69	6.69	6.69	6.69	20.34	20.34	20.34	20.34	20.34
Nitrogen (gm)	1.07	1.07	1.07	1.07	3.23	3.23	3.23	3.23	3.23
Urinary Nitrogen (gm)	3.50	3.48	2.97	2.66	2.77	2.91	2.77	3.33	2.77
Total Nitrogen Excretion (gm)	4.43	4.41	3.65	3.34	3.83	4.68	3.31	3.87	4.35
Nitrogen Balance	-3.40	-3.34	-2.58	-2.27	- .60	-1.45	- .08	- .64	-1.12
Creatinine Nitrogen (gm)	1.0	.91	.91	.91	.81	.81	.79	.62	
Creatinine (gm)	2.70	2.45	2.45	2.45	2.18	2.18	2.10	1.67	
Creatinine Nitrogen (%)	28	26	31	27	29	28	29	19	

'Accidental inadequate sampling

Food Intake and Nitrogen Excretions for Subject I
in the Four Experimental Periods

	Period III				Period IV			
	May 16	May 17	May 18	May 19	May 20	May 21	May 22	May 23
Weight	123.5	123	122.5	122	121.5	121.5	121.5	121.5
Intake: Total Calories	2152	2152	2152	2152	2158	2158	2158	2158
Carbohydrate (gm)	322	322	322	322	322	322	322	322
Fat (gm)	94	94	94	94	94	94	94	94
Protein (gm)	22.44	22.44	22.44	22.44	25.94	25.94	25.94	25.94
Nitrogen (gm)	3.88	3.88	3.88	3.88	4.12	4.12	4.12	4.12
Urinary Nitrogen (gm)	----	3.19	3.21	3.57	----	3.52	3.47	3.67
Total Nitrogen Excretion (gm)	----		4.19	4.55	----	4.92	4.28	4.48
Nitrogen Balance (gm)	----		- .31	- .67	----	- .80	- .16	- .68
Creatinine Nitrogen (gm)	----		.60	.60	----	.70	.71	.68
Creatinine (gm)			1.62	1.62		1.89	1.91	1.83
Urea Nitrogen (gm)	----	.34404	.69300	.98084	----	1.42511	.29699	1.25953
Ammonia Nitrogen (gm)	----	.045696	.098784	.085456	----	.183735	.112336	.139944
Creatinine Nitrogen (%)	----		19	17	----	20	20	19
Urea Nitrogen (%)	----	10.7	21.3	27.4	----	40.4	8.5	34.3
Ammonia Nitrogen (%)	----	1.4	3	2.4	----	5.2	3.2	3.8

'Accidental Inadequate Sampling

Table 6

Food Intake and Nitrogen Excretion for Subject II
in the Four Experimental Periods

	Period I					Period II			
	May 7	May 8	May9	May 10	May 11	May 12	May 13	May 14	May 15
Weight	131	131	131	131	131	131	131	131	131
Intake: Total Calories	1726	1726	1726	1726	1825	1825	1825	1825	1825
Carbohydrate (gm)	281	281	281	281	292	292	292	292	292
Fat (gm.)	64	64	64	64	71.1	71.1	71.1	71.1	71.1
Protein (gm)	6.69	6.69	6.69	6.69	20.34	20.34	20.34	20.34	20.34
Nitrogen (gm)	1.07	1.07	1.07	1.07	3.23	3.23	3.23	3.23	3.23
Urinary Nitrogen (gm.)	5.67	3.53	3.00	2.80	2.99	2.59	3.23	2.79	2.74
Total Nitrogen Excretion (gm.)	6.82	4.67	3.80	3.60	2.99	3.57	4.06	3.60	3.43
Nitrogen Balance	-5.75	-3.60	-2.73	-2.53	1.24	-.34	-.83	-.37	-.20
Creatinine Nitrogen (gm)	1.21	.74	.84	.60	----	.74	.74	.51	.25'
Creatinine (gm.)	3.23	1.99	2.26	1.62	----	1.99	1.99	1.37	.67
Creatinine (%)	21	21	28	21	----	29	23	18	9

' Accidental inadequate sample.

Food Intake and Nitrogen Excretions For Subject II
in the Four Experimental Periods

	Period III				Period IV			
	May 16	May 17	May 18	May 19	May 20	May 21	May 22	May 23
Weight	131	131	131	131	131	131	131	131
Intake: Total Calories	1862	1862	1862	1862	1868	1868	1868	1868
Carbohydrates (gm.)	295	295	295	295	295	295	295	295
Fat (gm.)	73.4	73.4	73.4	73.4	73.4	73.4	73.4	73.4
Protein (gm.)	22.92	22.92	22.92	22.92	24.42	24.42	24.42	24.42
Nitrogen (gm.)	3.64	3.64	3.64	3.64	3.88	3.88	3.88	3.88
Urinary Nitrogen (gm.)	——	3.24	3.02	2.35	——	2.67	3.23	3.42
Total Nitrogen Excretion (gm)	——	3.99	4.05	3.37	——	3.65	3.73	3.92
Nitrogen Balance (gm)	——	-.35	-.41	1.27	——	1.23	1.15	-.04
Creatinine Nitrogen (gm)	——	.51	.71	.64	——	.75	.75	.83
Creatinine (gm.)	——	1.37	1.91	1.72	——	2.02	2.02	2.23
Urea Nitrogen (gm.)	——	.07327	.61404	.57720	——	.36278	1.07352	.314720
Ammonia Nitrogen (gm.)	——	.136136	.07476	.101472	——	.113288	.06552	.01008
Creatinine Nitrogen (%)	——	16	24	27	——	28	23	24
Urea Nitrogen (%)	——	2.2	20.3	24.5	——	13.2	33.2	9.2
Ammonia Nitrogen (%)	——	4.2	2.4	4.3	——	4.2	2	.3

Table 7

Comparison of the Average of Different Forms of Nitrogen Excretion of Experimental Subjects with Averages Found by Beard

Low Protein Diets

				Subject I					
	Beard			Period III			Period IV		
Volume of Urine	1408 cc			1278 cc.			1677 cc.		
Specific Gravity	1.019			1.018			1.016		
Substance	Amount	Nitrogen Content	Per cent of Total Nitrogen	Amount	Nitrogen Content	Per cent of Total Nitrogen	Amount	Nitrogen Content	Per Cent of Total Nitrogen
	(gm.)	(gm.)		(gm.)	(gm.)		(gm.)	(gm.)	
Total Nitrogen	....	7.97	....	....	3.32	....	....	3.55	....
Urea	13.20	6.16	77.29	1.44	.672631	20.26	2.13	.99388	28.1
Ammonia	0.52	0.43	5.39	.17	.076979	2.32	.18	.145338	4.1
Uric Acid	0.54	0.18	2.26	....	....	....	....	....	....
Creatinine	1.67	0.62	7.78	1.62	.60	18.00	1.88	.70	19.7
Undetermined nitrogen by difference.....	....	0.58	7.28	....	1.97	59.33	....	1.71	48.2

Table 8

Comparison of the Average of Different Forms of Nitrogen Excretion of Experimental Subjects with Averages Found by Beard

Low Protein Diets

	Subject II								
				Period III			Period IV		
Volume of urine	1408 cc.			1427 cc.			1824 cc.		
Specific Gravity	1.019			1.015			1.014		
	Amount (gm)	Nitrogen Content (gm.)	Per Cent of Total Nitrogen	Amount (gm.)	Nitrogen Content (gm.)	Per Cent of Total Nitrogen	Amount (gm.)	Nitrogen Content (gm.)	Per Cent of Total Nitrogen
Total Nitrogen	.....	7.97	.....	.....	2.87	.....	.....	3.11	.....
Urea	13.20	6.16	77.29	.9	.4215	14.69	1.18	.55034	17.69
Ammonia	0.52	0.43	5.39	.13	.104123	3.63	.08	.062963	2.02
Uric Acid	0.54	0.18	2.26	.....	.....	.....	.....	.....	.....
Creatinine	1.67	0.62	7.78	1.67	.62	21.50	2.10	.78	25.08
Undetermined nitrogen by difference.....	.....	0.58	7.26	.....	1.73	60.18	.....	1.72	55.30

SUMMARY AND CONCLUSIONS

Total nitrogen intake and excretion determinations were made on two subjects in four feeding periods of low protein intake (1.5 -5.2%). Total nitrogen determinations were made by the Kjeldahl method.

In the fourth period coffee was added to the diet of the third period and the nitrogen partition products of the urine compared for the two periods.

Creatinine was determined by Folin's Modification using the photoelectric colorimeter. Urea and ammonia were determined by the Van Slyke and Cullen method.

The data from this study reveal the following:

1. When subjects were given a low protein diet, the resulting total nitrogen excretion and negative balance decreased progressively each day.

2. Balance was easily obtained for the subject given the lowest caloric intake, with 65 -59.5% of the calories furnished by carbohydrate, 33 -35% by fat, and 1.5 - 5.2% of protein.

3. True nitrogen balance was not obtained for the subject on the higher caloric intake, with 65 -56% of the calories from carbohydrate, 33-39% from fat, and 1.5 - 4.8% from protein.

4. Balance was reached by the subject on a caloric intake of 1862 calories, with 22.92 gm. protein largely from milk and eggs, or with 24.42 gm. protein when the coffee nitrogen is added.

5. Coffee had no noticable effect on total nitrogen excretion or on the nitrogen balance.

6. The creatinine output on the whole was relatively stable.

with the exception of several days in which accidently inadequate samples were obtained.

7. The urea gm. and percentages were very low compared with other studies.

8. With the addition of coffee to the diet a small average increase in urea output was observed with both subjects. (8% and 3%)

9. With the addition of coffee to the diet an increase in the average output of ammonia was noted for one subject (1.6%) and a decreased (1.4%) output for the other subject.

The figures in this study for urea and ammonia excretion are known to be unreliable. Further experimentation with the urea-ammonia apparatus has shown that the rubber tubing was not always completely airtight. In making this apparatus, it was impossible at the time to secure new tubing of the proper size.

The time suggested by Hawk and Bergeim (Physiological Chemistry) for the action of the enzyme is definitely inadequate for a quantitative determination of urea. It was found that the enzyme, with optimum temperature and pH, should be allowed to stand for at least 1 hour before adding the potassium carbonate and aerating for 30 minutes.

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APPENDIX

REAGENTS (SOLUTIONS)

All reagents were of the highest quality and as free as possible from nitrogen.

0.02 Normal Oxalic Acid:

1.26 gm. oxalic acid
Distilled water to make 1 liter.

0.02 Normal Sodium Hydroxide:

.8 gm. sodium hydroxide
Distilled water to make 1 liter
Check against 0.02N oxalic acid using phenophthalene.

0.02 Normal Hydrochloric Acid:

1.8 ml. concentrated hydrochloric acid
Distilled water to make 1 liter.
Check against 0.02 N sodium hydroxide using methyl red.

0.1 Normal Sodium Hydroxide:

4 gm. sodium hydroxide
Distilled water to make 1 liter.

0.5 Normal Hydrochloric Acid:

45 ml. concentrated hydrochloric acid
Distilled water to make 1 liter.

Saturated Picric Acid:

1 liter distilled water
Excess of picric acid.

Saturated Potassium Carbonate:

500 ml. distilled water
Excess of potassium carbonate.

Buffer Solution:

6.2 gm. boric acid
7.5 gm. potassium chloride
35.5 ml. of 0.2 N. sodium hydroxide
Distilled water to make 500 ml.

Urease Solution:

Eimer and Amend Urease Solution number So-U-26.

Standard Creatinine Solution:

Eimer and Amend standard creatinine solution.