THE DIETARY PROCEDURES EMPLOYED IN CONDUCTING A NON- CLINICAL METABOLIC BALANCE STUDY

Thesis for the Degree of M. S.

MICHIGAN STATE UNIVERSITY

Carolyn Mae Friedemann

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ABSTRACT

THE DIETARY PROCEDURES EMPLOYED IN CONDUCTING A NON-CLINICAL METABOLIC BALANCE STUDY

by Carolyn Mae Friedemann

Non-clinical metabolic balance studies have long been employed to evaluate the nutritional requirements of human beings. The dietary procedures to be used in such studies have not been well defined. A non-clinical metabolic balance study in which the dietary procedures were evaluated was undertaken to investigate bread as a primary source of protein for man. This paper concerns only the dietary procedures involved in such a study. A later presentation by Miss S. Bolourchi will discuss the clinical aspects of the study.

The subjects chosen for the experiment were twelve male college students. The control period and the experimental period were twenty-one and fifty-one days in length, respectively. The amount of protein in both diets was limited to approximately 65 grams per day. During the control diet small amounts of animal protein were served, whereas, in the experimental regimen no animal protein was allowed. In the experimental diet 90 to 95 per cent of the protein was derived from wheat products.

A seven day menu cycle was devised for each period. Tables of food composition were used to calculate the nutrients in the diets. A diet which was a duplicate of those served to the subjects was collected each day for analysis. Nitrogen, fat, calories, and moisture were determined in the dietary samples.

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The diets contained mixed dishes and desserts such as cake and pie. These increased palatability and acceptability of the diet consumed during the experimental period. To provide an extra source of calories, foods such as hard candies were provided on an ad libitum basis. The intake of these was permitted only as a supplement to the regular diet. The amount of these foods consumed was measured.

The food purchased was of the same variety and brand throughout the study. All non-perishable items were purchased in sufficient quantity for the study. The perishable items were purchased biweekly. The non-perishable items were stored in the dry storage areas, whereas, the perishable items were stored in a refrigerated walk-in or a freezer.

To be certain that the diet collected for analysis was representative of the diet the subjects consumed, the samples were collected when the majority of the subjects were served.

Upon comparing the calculated values for the diets to the analyzed values, tables of food composition were not reliable for fat. They are accurate for protein. The nitrogen was calculated from the computed protein values with the aid of the specific conversion factors for each food group. The calculated values for nitrogen were within 5 per cent of the analyzed values in 71 per cent and 86 per cent of the control and experimental diets, respectively. The diet analyzed in toto was in better agreement with the calculated values for nitrogen than when the individual items of the same diet were analyzed and totaled.

The differences between the analyzed and calculated calories in the experimental and control diets were statistically significant.

THE DIETARY PROCEDURES EMPLOYED IN CONDUCTING A NON-CLINICAL METABOLIC BALANCE STUDY

By

Carolyn Mae Friedemann

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TABLE OF CONTENTS

TABLE OF CONTENTS (Cont.)

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LIST OF TABLES

LIST OF TABLES (Cont.)

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LIST OF FIGURES

REVIEW OF LITERATURE

A metabolic balance study will be defined as an investigation of the intake and output of a nutrient or nutrients by human beings. This can occur by keeping constant all possible factors other than the one that is being explored(Comfort, 1957). Such a study requires the precise control and measurement of both intake and output. Because this is such a vast subject, this review of literature will consider only the first phase; that of intake. This presentation is divided into three sections. The first section will concern the general principles involved in conducting a metabolic balance study; the second will present the factors involved in the dietary management of such a study; the third will compare the two methods employed to determine the nutrient value of the diets.

I. General Considerations

Clinical versus Non-Clinical Balance Study

A metabolic balance study may be carried out in a clinical or a non-clinical situation. In the former there is constant supervision of the subjects, whereas, in the non-clinical study the only supervision the subjects receive is generally during meals and the various tests to which they are subjected. The site of the clinical study is the hospital, whereas, the university or a comparable institution composes the non-clinical location. Bauer and Aub (1927), are the forerunners of today's modern research metabolic unit in a hospital. They stressed that the uniqueness of such study is the control of all phases involved. Since there is more supervision in a hospital, there is inevitably increased control. Coons (1930) conducted a metabolic study, using as her

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subjects pregnant women who were consuming a self-chosen diet in their homes. The only supervision entailed in this study was visits made to the individual's home during the serving of the meals. Less control of the study occurs, and thus more variables enter into the non-clinical study.

There are a number of factors which further distinguish a clinical study from a non-clinical study. One of these is cost. The clinical situation is more expensive than the non-clinical. The difference in cost may be a factor ranging from four to five fold depending on the location of the study. Furthermore, in most cases, the subject in a clinical situation cannot perform physical activity at a normal level. Since physical exercise influences the deposition of fat and the development of muscle, a lack of it could play an influential role in the results of the study. The application of these findings to normal adults might thus be questioned. The third comparison of the two sites for a metabolic balance study is the psychological aspects of the environment. In the hospital the emphasis is primarily upon illness. The environment created in a non-clinical situation is more typical of home life. McKay et al.,(1942) report that it is important that the participants maintain their usual curricular and extracurricular activities and continue their ordinary manner of life during a balance study.

Schottstaedt and co-workers (1958) conducted a study to determine the effects of naturally occurring stressful situations on a group of patients in a social setting of a metabolic ward. It was discovered that there were metabolic changes in the urinary excretion of water, sodium, potassium, calcium, nitrogen and creatinine during situations of stress. Interpersonal difficulties were the most common source of

tension which produced the metabolic changes. They concluded that a metabolic ward is not necessarily a natural environment, but adequate interpretation of metabolic deviations must include an appraisal of the participant's life situation and emotional state at the time of the experiment. Thus, for normal adults the clinical study is limited and, consequently the results may be of limited value.

Objectives of the Study

The objectives of a metabolic balance study are defined as the questions which the study plans to answer. The objectives of the study determine its length which includes the control and experimental periods, the requirement of a transitional period between the control and experimental regimens, the number of personnel needed to carry out the study (Horwitt et al., 1949), the selection of the subjects (Leichsenring et al., 1958), the choice of foods, the method of food cookery, the type of storage required for the food, the composition of the menu or menus (Sampson et al., 1952), the precautions to be employed in collecting the food for analysis, and the chemical determinations to be performed.

The researcher in designing the objectives should take into consideration money and personnel available to conduct the study (Leichsenring et al., 1958). These two factors influence the number of subjects, the number and/or type of analytical methods to be employed, the menus and the preparation of the food.

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Experimental Design

Control and Experimental Periods

A metabolic balance study should be composed of two periods. These two periods are control and experimental. The control period is the phase during which the subjects are becoming adapted to the procedures involved in a balance study, the dietary regimen and to the intake of the nutrient that is going to be studied in the experimental period. The control period should serve as a period in which the subject and the investigator develop rapport.

In addition to the preceding, Leichsenring et al., (1958) suggest that a trial period be carried out prior to the study in which everything but the analytical work is performed. They claim that such a period helps to clarify procedures and also assists in developing a rapport between the researcher and the subject. However, since the control period serves as the phase during which the subjects are attaining equilibrium of the nutrient to be investigated, it could also serve as the trial period. The results of the control period serve as the norm with which the values from the experimental period are compared. The two cycles may either occur simultaneously or sequentially.

Reifenstein et al., (1945) and Comfort (1957) report that a metabolic balance study may follow one of two plans. The first plan compares the finding of one individual who is on a control diet with the results of another subject who is on an experimental diet. The second arrangement consists of a comparison of the results of a subject when he is on a control diet with the results of the same subject when he is on an experimental diet. Another experimental design is reported

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by Bricker et al., (1949). In this plan half the subjects are started on the control diet and the other half on the experimental diet. Half way through the experiment, the regimens for the two groups are switched. By this means, it is possible to compare the results for the same subject during the experimental and control periods. It is furthermore possible to compare throughout the entire duration of the study, the subjects on the experimental regimen with those on the control. This also, provides a control for any environmental factors that might influence the study and which might change during its course.

There are other experimental designs for a balance study which are not cited in the literature but which require discussion. The first involves the use of two separate groups; the first is maintained on the control diet throughout the experiment; the second group is on the control diet to establish equilibrium and then proceeds to the experimental diet. The results of the experimental diet are compared with the control group. The advantage of this design can be explained by an example. In the bread study conducted at Michigan State University, Spring, 1964, there was a weight loss in most of the subjects during the experimental period. In this experiment the results of a subject on the control diet were compared with the results of the same subject on the experimental regimen. If a control group had been employed simultaneously with the experimental group, it would have been possible to determine with considerable certainty whether the weight losses experienced by the subjects were due to the increased physical activity which presumably occurred with the advent of warm weather. (See Section Results and Discussion, p.71). However, for such a design additional personnel and facilities for food storage, preparation and serving are required. The two groups should be served in separate areas

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Another experimental design involves the use of an experimental diet which is preceded and followed by a control regimen.

Transitional Period

Reifenstein et al., (1945) report that if the same subjects on the control diet are placed on the experimental diet a transitional period between the two phases may be required. This is only the case if the control diet is high in the substance which is extremely low or absent in the experimental diet. The analytical results obtained when the subject first starts on the experimental diet should not be considered as conclusions for the experimental phase. A lapse of time must occur before a new equilibrium is established (Reifenstein et al, 1945). Coons (1930) reports that during this period no collections of excreta or diets should occur. This length of time has been reported to be three to five days (Coons, 1930). However, the exact length of time depends on the difference in the nutrient content of the two diets. A fifteen day period preceded the ten day collection period in studies conducted by McKay et al., (1942). This study was concerned with the effect of different levels of milk upon the calcium, phosphorus and nitrogen metabolism in college women. They found this length of time necessary in order for the women to establish equilibrium on the experimental regimen.

Length of the Study

The length of a balance study is very important because of the extreme daily variations manifested in the magnitude of retention or loss of the various nutrients (Donelson et al., 1931). It is further reported by Donelson et al., (1931) that a balance study which includes

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only a few days registers fluctuations in metabolic processes which a longer period tends to conceal. Both periods should be sufficient in length for the subjects to attain equilibrium for the nutrient under consideration. The length of the control period is influenced by the amount of change between the control regimen and the diets of the subjects prior to the study.

Collection Period

Within both phases, control and experimental, of a balance study there are collection periods. The length of this period usually varies according to the number of menus designed for each period. Young et al., (1953) in studying reducing and post-reducing maintenance on a moderate fat diet employed a seven day diet, thus, a seven day composite period. A composite sample represents the total collection for the balance period. This would be made up separately for food, urine, and feces. A six day collection period was used in studying the metabolic patterns in preadolescent children (So. Coop. Series Bull. No. 94, 1964). Schofield et al., (1956) employed a four day collection period. Bauer and Aub (1927) used a three day collection period in their studies of inorganic salt metabolism. In each of the studies previously mentioned, the number of menus was equal to the number of days in the collection period.

Number of Subjects

No report was found in the literature that describes the ideal number of subjects to be employed in metabolic balance studies. The number of subjects range from one (Sherman, 1920) to one hundred twenty-four (McKay et al., 1942). The extent to which they represent a sample of the normal population has not been discussed by the investigators.

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Selection of the Subjects

The emphasis of the study will determine the age, sex, health, and socio-economic status of the subjects to be selected (Leichsenring et al., 1958). At a university today, varied cultural backgrounds which mean assorted dietary habits may play a more important role than the socio-economic status. The subjects with food dislikes or allergies which would interfere with their complete consumption of the diets should be eliminated. The researcher must also consider such characteristics as the integrity, reliability and the native ability of the individual (Leichsenring et al., 1958). The subject prior to selection should have a thorough physical examination to assess his general health and nutritional status (Leichsenring et al., 1958). The subject should be free of disease. These are broad generalizations which have to be supplemented by specific instructions to the examining physician. Individuals with physical handicaps should be omitted from any balance study which attempts to evaluate normal individuals. The subjects selected should be as uniform as possible insofar as their body weight is concerned. For normal individuals an adherence of 5 per cent of their ideal weight should be followed. In addition to height-weight tables for evaluating a subject's weight, a researcher may wish to employ anthropometric measurement such as those of skin folds.

Upon selection, the subject should be presented a clearly written statement which includes his obligations, the directions for the collection of excreta, and if applicable, the directions for the collection of food (Leichsenring et al., 1958). Preceding the study the research director should obtain each subject's permission to enable him to use any data as a result of the investigation (Leichsenring et al., 1958).

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Horwitt et al., (1949)selected their subjects according to the following procedure. They first selected 50 per cent more subjects than were required by the study. The subjects were observed for two months on a dietary regimen similar to that which was to be employed in the study. They evaluated the subjects' attitudes, cooperativeness, and eating habits. The individuals deficient in any of these respects were eliminated. This study was conducted in a mental hospital which makes this arrangement possible.

To eliminate subjects who may have emotional problems to the extent that they would be unreliable, irresponsible, and dishonest, Young, (personal communication, 1964), suggests the use of the Bell Adjustment Inventory Examination[1]. The total score of the Stanford examination is divided into four parts--home, health, social, and emotional. Young indicates that before subjects are selected for her metabolic balance studies they must score an excellent or good emotional score. The selection of the participants on the basis of the emotional score eliminates those who are not able to withstand the restrictions that are involved in a controlled feeding experiment (Young, personal communication, 1964).

Menus and Diets

The control and experimental diets should contain the same components with the exception of the nutrient that is to be investigated during the experimental phase. Furthermore, since the control period serves as the time when the subject is becoming adapted to the dietary

[1] This is a publication of the Stanford University Press and is distributed by the Psychological Corporation, 522 Fifth Avenue, New York 18, New York. The examination is available in two forms, an adult form and a student form.

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regimen, the two diets should contain similar types of food insofar as possible. For example, if the balance study is to evaluate a low thiamine intake on college men, the control diet should be the same as the experimental regimen. The experimental diet would require supplementation in order for the diet to fulfill the Recommended Dietary Allowances (National Research Council, 1964). Ideally, the only difference between the two diets would be the change in the amount of thiamine supplementation during the experimental diet.

The diets and menus are further discussed in the following section (II. Dietary Management).

The Personnel

In reviewing the literature regarding the personnel required to conduct a balance study, there is only slight reference to this topic. The people that are employed for any phase of the study must be intelligent, willing to follow detailed instructions precisely and be accurate in their work performance (Leichsenring et al., 1958). The number of personnel required to supervise thirty subjects in a study to determine the riboflavin requirements of adult subjects, included a dietitian, two cooks, and a nursing attendant or equivalent (Horwitt et al., 1949). This paper did not report the number of employees used to perform the analytical techniques as demanded by the balance study.

Evaluation of Results

This discussion will be limited to the results of the nitrogen balance studies. In any balance study the researcher is faced with the intake and output of a specified nutrient which is relatively large when compared to the amount retained. Very few investigators have carefully evaluated the over all significance of positive nitrogen equilibrium.

Leverton et al., (1956) report that because of the presence of inherent variations, both human and mechanical, in human metabolism studies an evaluation of the results require careful consideration.

A nitrogen equilibrium is defined as a zone in which the excretion and intake closely approximate each other, rather than a single point at which they are numerically identical (Leverton et al., 1956). Therefore, in their studies of amino acid requirements, the subjects were in nitrogen equilibrium when the difference between the intake and excretion did not exceed 5 per cent.

Rose (1957) comments that irregardless of the accuracy in controlling the amount of nitrogen that a subject consumes there is a fluctuation of nitrogen from day to day. Perhaps the explanation is a result of slight alterations in the daily rate of metabolism or excretion or in the degree of muscle tone (Rose, 1957). In establishing the amino acid requirements of human adults, Rose (1957) induced a negative nitrogen balance. The intake of the amino acid under investigation was then increased until a slight but distinct positive nitrogen balance, as measured by the average for a period of several days, was achieved. The greatest amount required to achieve the positive balance in an individual was defined as the amino acid requirement for normal man.

Walker (1962) states that the results of nitrogen balance studies may reveal an excessive accumulation of nitrogen which over a prolonged period of time would be an impossibility. This may be due to a loss of the nitrogen other than through the urine and the stools, especially losses through the skin and in certain circumstances significant changes in body composition. This fallacy was illustrated by Walker (1962) from a long-term balance study on malnourished Bantu adults

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conducted by Holmes (1954). The observations from this study disclosed a consistently high retention of nitrogen which was, in some cases, as much as ten grams per subject per day. The diet contained 100 grams of protein and 3000 to 4000 calories per day. Occasionally the protein content was increased to 200 grams per day. Such retentions were accompanied by much lower gains in body weight than is to be expected if the subject was storing ten grams of nitrogen daily.

There are three sources of error that may influence the nitrogen balance of an individual (Walker, 1962). They are (1) inaccuracy in intake and output collections and methodological errors, particularly in long-term balance studies which are liable to lead to cumulative errors, (2) lack of knowledge regarding losses thru the skin and other sources, (3) lack of methods for precisely determining body composition.

II. Dietary Management

The choice of foods for a metabolic balance study is determined by the facilities available for proper storage, season of year, method of preparation, and purpose of study.

Purchase and Storage

Sampson *et al.*, (1952) suggest that the entire lot of food with the exception of fresh food be purchased from a single source in quantity sufficient for the duration of the study. This requires adequate storage space. The freezer space per subject is a minimum of five cubic feet.

As often as is possible and facilities permitting, all food items should be prepared at one time and frozen until needed (So. Coop. Series Bull. No. 94, 1964). The diets described in this bulletin contained such items as bread, fruit-flavored ice, cupcakes,

and other desserts. Prior to the experiment, these items were purchased and/or prepared for the length of the study. The designated portions of the items were weighed and stored in a freezer.

The food selected for a balance study should undergo minimal change in composition during storage (Sampson et al., 1952). The canned or frozen foods are not altered significantly in composition if stored properly, however, upon storage perishable items are changed. If the determination of vitamins is the objective of the study, the perishable items should be stored for short periods of time (Sampson et al., 1952). Hawks et al., (1937) state that perishable foods should be purchased for one balance collection period. This eliminates the variability in composition which occurs in food when purchased on a day to day basis.

Marble (1939) reports that all food used should be in season throughout the experiment. This statement is most important in selecting fresh foods. However, this factor would also apply to canned or frozen food if there is inadequate storage space. This problem can be alleviated by ordering the same lot and brand of food in adequate amount at the beginning of the study and arranging with the food supplier or the proprietor of storage facilities for storage of the items until needed.

Number of Menus

The number of menus employed in a balance study usually is the same as the number of days in the collection period. Presson (1955) suggests that a trial period prior to the actual study should be employed to determine the most palatable diet for the subject. The use of one

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diet according to Presson (1955) eliminates excessive calculations and errors which may occur when using many menus. The use of one menu is ideal, but is not always possible. In order to achieve a better definition of intake Ahrens et al., (1954) advises the use of only one to three menus. Bassett and Van Alstine (1935) report that one menu may be well tolerated by children but, frequently, when presented to adults leads to refusal and smuggling of food. To achieve palatability and acceptability of the diet by the subjects in their studies, a six day menu was required. A factor to be considered is that their study was four months in length. Any study of this duration increases the non-acceptance of one menu, whereas, for short term studies a one day menu is more likely to be tolerated.

Composition of the Diet

The composition of the menus should not only consider the purpose of the study but also, the subject's eating habits and caloric requirements (Sampson et al., 1952). The nutrient content of the diet is calculated with the aid of food composition tables. All calculated menus should insure an adequate amount of all the essential nutrients. If the diet does not fulfill the nutritional requirements, supplements in pill form which contain the deficient nutrients should be given the subjects. Food composition tables should only be used as a guide in planning the diets (Widdowson and McCance, 1943). Because of this factor Coons (1930) emphasizes that all diets require chemical analysis prior to the beginning of the study.

Selection of the Menu

To determine the eating habits of the participants the researcher should interview the subject. This information can aid in determining

the foods which are to be included in the menu (Presson, 1955). Sampson and co-workers (1952) advocate the participant's selection of the menu. This avoids the continual encouragement that must be given to the subject by the nutritionist when the subject does not desire the food. The emotional disturbances created by forcefully eating an objectionable food could contribute to unphysiological results. If the subject does not consume the food, the results of the balance study would be affected. Marble (1939) stresses that unconsumed food must be recorded. Subjects with many food dislikes should be eliminated. This is only possible when there is a large number of subjects from which to choose.

Caloric Requirement

The calories in the diet can be calculated according to the regular dietary intake of the subject if the study is concerned with the maintenance of the subject's weight. Also, the caloric requirement of the individual may be determined according to age and sex (Sampson, et al., 1952).

To fulfill the energy requirements of the participants whose needs were not met by the calculated diet Lang et al., (1965) served hard candy and butter balls ad libitum. Their studies were to determine the manganese metabolism of college men. To supply additional calories to subjects on controlled caloric intake Pilcher (1961) administered sugar, honey, butter, salad oil, jelly and jam.

Food Preparation

Johnston and McMillan (1951) in preparing mixed dishes weighed the ingredients in individual casseroles prior to cooking. The objective of their study was to investigate the effect of spinach on the absorption of iron. Because the study was concerned with iron, all the

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Bauer and Aub (1927) and Gephart and DuBois (1915) stressed the importance of weighing, cooking and serving the food in the same dish. In their clinical metabolic balance studies they recorded on the dish with a moisture resistant enamel pencil the weight of the empty dish. Any food not consumed by the participants was determined by reweighing the dish plus its contents. The weight of the uneaten portion was obtained by difference.

In addition to weighing, cooking and serving the food in the same dish, Hawks et al., (1937) rinsed the dish with distilled water after its contents had been consumed. This water solution is then consumed by the participant. Hummel et al., (1942) stressed that the cooking water should not be discarded in mineral balance studies or in those studies where vitamins are to be determined. The importance of Hawks et al.,(1937) and Hummel et al., (1942) suggestions may be questioned. These factors are not important as long as the sample collected for analysis is treated in the same manner as the diet which is consumed by the subjects. Furthermore, the consumption of pot liquor might be objectionable to the subjects.

Distilled water, according to Coons (1930), should only be employed in those studies investigating minerals. If distilled water is not used, the tap water should be analyzed for the minerals of importance in the study. For studies involving mineral determinations the water intake of the subjects should be recorded.

The mode of food preparation and cookery used for a balance study is very important to assure uniformity and consistency of the product. Since the cooked products should have no variable loss of nutrients, steaming has been recommended for food preparation (Marble 1939).

Marble (1939) does not state the type of balance studies which would require steaming. For mineral and vitamin studies this method might be employed. However, if the diet sample for analysis is a duplicate of the diet consumed by the subjects, the method of cooking need not be limited to steaming. If the nutrient under investigation is not destroyed by most cooking procedures, the choices for food preparation are greatly increased.

In using special recipes precautions are necessary to avoid variations in content of water and solids. This is best achieved by meticulous measurement of ingredients, standardization of cooking time and temperature (Sampson et al., 1952). It was further suggested that desserts, such as cake and pie, should not be used in balance studies because of their inevitable variation in composition

In conducting a nitrogen balance study there is an increased versatility in the selection, in the type of storage, and in the cooking procedures of the foods. This does not mean less control, for the results of any balance study are only as reliable as the techniques employed in conducting the study (Hawks et al., 1937). If a vitamin, such as ascorbic acid, is being investigated, food must be selected which is not perishable and all cooking methods must insure minimal variability in the losses of the vitamin.

Calculation of Mixed Dishes

If mixed dishes are to be employed in the study, the recipe should be calculated. Accurate results cannot be attained by using for mixed dishes the values listed by tables of food composition (Patterson and McHenry, 1941). This is due to the different proportions of the

ingredients in the recipe as presented by the tables and in those designed for a balance study.

Weighing and Measuring the Food

Widdowson and McCance (1942) and Coons (1930) weighed their diets on a Hanson spring balance. They report that the scale was accurate to one gram. Contrary to their work, Sampson et al., (1952) and Lang and co-workers (1964) employed a torsion balance for weighing all food items. The torsion balance was accurate to 0.1 gram. Ceglarek et al., (1958) measured liquids to the nearest ml.

Formula Diets

Because of the difficulty in controlling the variability when using assorted natural foods, formula diets have been suggested by Ahrens et al., (1954). For specialized metabolic studies, the formula diets may be altered to give unusual proportions of fat, protein, and carbohydrate. A formula diet can be devised to contain relatively few components of definite composition which can be accurately formulated, homogenized, and dispensed (Haust and Beveridge, 1958).

Many researchers using the formula diets have studied the effect of various kinds and levels of fat upon plasma lipids. An example of this type of formula diet is the one used by Beveridge and his co-workers (1955). The ingredients of the basal diet were powdered skim milk, corn oil, margarine and dextri-maltose. When other fats were substituted, the sugar was used to adjust the calories to a constant amount. Vitamins for the subjects were supplied in the form of capsules and roughage was provided by the addition of celluflour to each formula.

Ahrens (1954) fed formula diets to adults for periods of two to sixteen weeks. The formulas for all subjects proved to be entirely

acceptable, economical and simple to use. He suggests the use of formula diets for those studies concerned with maintaining the weight of the subjects. This can be obtained by increasing the caloric intake without disturbing the composition of the diet. Formula diets can also be used when the calories are to remain constant but the proportions of carbohydrate, protein, and fat are to be varied or when the proportions of these three are to remain constant.

Dietary Analysis

Reifenstein et al., (1945) and Bassett and Van Alstine (1935) report three procedures for the analysis of the diets. They are: (1) each foodstuff may be analyzed and the values obtained may be used in the calculation of the diet, (2) one or more sample diets may be analyzed in toto and the results obtained may be employed throughout the study or (3) aliquots of the diet, as served each day, may be pooled and analyzed for each collection period of the study. They report that it is most accurate to sample and analyze the food from each collection period, however, due to the increased labor and time requirements, they suggest the second method. In the studies of Sampson et al., (1952) the diet is reanalyzed every time a new lot of any food is used. Presson (1955) also advocates the occasional analysis of the total diet or diets employed in a study. The values obtained are used to determine the actual intake by the subjects. Lutwak et al., (1964)determined the composition of seven diets by collecting and analyzing a representative sample every fourth week. Hunter et al., (1948) reports that the assessment of diets by direct analysis immediately before ingestion is the best means of determining the nutritive value. Assuming accuracy in the collection of the diets, and also assuming reliability of the

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analytical techniques used for measuring the food constituents, the direct analytical method can yield a high degree of accuracy (Hunter et al., 1948).

Collection of the Sample

The collection of the sample for analysis must be performed in a constant manner throughout the study. It is reported by Coons (1930) that the collection of the sample for analysis should occur at the time that the item is served in order to avoid changes in moisture content. She reports that weighing the sample which is to be collected requires a torsion balance accurate to 0.1 gram. It is further reported by Coons (1930) that the sample should be homogeneous. Hawks et al., (1937)state that it is impossible to obtain a homogeneous sample of food for analysis even under strictly controlled conditions. The principle to adopt for accurate sampling as presented by Widdowson and McCance (1942) is to weigh each ingredient separately or to make a mixture of the components so that an average helping is likely to be representative of the whole dish.

The amount of sample weighed for the analysis of the diet depends upon the number and type of chemical determinations that are required. Johnston and McMillan (1952) support the principle of collecting the same amount of food that is served to the subjects. Bassett and Van Alstine (1935) and Leichsenring et al., (1958) weigh one-fourth of the prescribed weight of each item served.

Components of the Sample

The exact foods which will constitute the collected sample varies with the chemical determinations employed in the study. High fat diets and high sugar diets are often difficult to handle after drying (Coons, 1930). Leichsenring et al., (1958) and Reifenstein et al., (1945)

suggest that butter, fat, sugar and other fats be excluded from the collected diet sample and analyzed separately. If fat and calorie determinations are required as a part of the study, the fatty foods cannot be omitted (Leichsenring et al., 1958). If only mineral and nitrogenous components are being determined, Leichsenring et al., (1958) advocates weighing a proportional aliquot of the fat items. These fatty foods are heated with 50 to 100 ml. of distilled water until the fat is melted. Upon cooling the layer of solidified fat is removed and the remaining portion is then analyzed. The nutrients which are to be investigated by the study must be considered prior to employing the previous technique. For example, if the determination of phospholipids or phosphorus was an objective of the study, this method could not be practiced.

The division of the diet into two portions is practiced by Gutman and Low (1939). The samples containing the nutrients which are subject to rapid deterioration are analyzed immediately, whereas, the samples composed of stable nutrients are made into a slurry and frozen in a low temperature refrigerator. No further description of the refrigerator was presented.

Leichsenring et al., (1958) state that the number of analysis may be reduced by preparing composites of several foods having similar physical and chemical properties. In studying the protein requirements of women on high cereal diets Bricker and co-workers (1949) employed four divisions. These were: non-protein dessert which was cornstarch cookies, low protein foods such as fruits and green vegetables, cereal protein, and non-cereal protein such as potatoes, meats, and animal

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products. The objectives of that study were to determine the protein requirements of college women when consuming various nitrogen levels from different sources. The samples were pooled as composites for the particular length of the balance period.

Containers for the Collection

Leichsenring et al., (1958) report that wide-mouthed French-square glass bottles with plastic screw caps are satisfactory for collecting the diet because they can be compactly stored. Vought and London (1964) in determining the iodine intake of subjects living at home employed one gallon, wide-mouthed, polyethylene jars for the collection of the diet. The liquid and solid components of the diets of lactating women were collected individually in glass containers by Kaucher et al., (1945).

Weight of the Diet Sample

Upon placing the prescribed amount of food in the container, it should be covered immediately and placed in the freezer (So. Coop. Series Bull. No. 94, 1964). The weight of the container and the date of the sample should be recorded on the container with a moisture resistant pencil (Leichsenring et al., 1958). Bransby et al., (1948) upon collecting the entire diet, weighed the jar with its contents. The weight of its contents was the difference between the two weights and was recorded on the container. The weight of the diet was obtained after homogenization in the diet studies on preadolescent children (So. Coop. Series Bull. No. 94, 1964).

Preparation for Analysis

Upon collection of the diet it is homogenized in a Waring blendor. Whenever needed, water is added to bring the mixture to the desired consistency (Bransby et al., 1948). The amount of water added should

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be measured accurately and recorded. Other investigators suggest bringing the slurry to a constant weight (McKay et al., 1942) or volume (Wharton et al., 1953).

When a composite is collected for a balance period, a portion of the homogenate is saved each day until the period is completed. These portions are then thoroughly homogenized and then apportioned in duplicate for the various analytical determinations. In the studies on preadolescent children a separate aliquot was removed for each of the following--energy, nitrogen and fat (So. Coop. Series Bull. No. 94, 1964).

For nitrogen and mineral measurements the diet composites may be refrigerated, frozen, or converted into digests (Leichsenring et al., 1958). Special manipulation of samples is required where food energy values or amino acid contents are to be determined or where labile compounds such as thiamine, riboflavin or ascorbic acid are to be measured. For light-sensitive nutrients the diet samples should be composited and aliquoted in darkened laboratories (So. Coop. Series Bull. No. 94, 1964).

Storage of the Sample

The sample may be analyzed immediately following homogenization or it may be stored for future analysis. If the latter is the case, the sample must be stored with precautions so that no objectionable chemical changes occur in the diet. Leichsenring and co-workers (1958) suggest the use of pharmaceutical bottles or round glass bottles, wide or narrow-mouthed. Amber colored bottles are employed for storing samples when analysis for light-sensitive nutrients are to be made. Plastic screw caps are inert to most chemicals and can be used to preserve samples, although in the presence of toluene and solvents of

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like nature, a cardboard interliner may be used (Leichsenring et al., 1958). Leichsenring and co-workers(1958) also advise that because metal screw caps easily rust, precautions should be taken if they are to be employed. Generally, a piece of cellophane or aluminum foil placed over the mouth of the jar prior to covering will provide sufficient protection. McCay et al., (1945) stated that storing the homogenized sample in a cellophane bag in a freezer protects the sample for several weeks without decomposition. Pilcher (1961) utilized jars and cartons for storing the homogenized samples in the freezer.

The temperature at which the samples should be stored is -20° C (So. Coop. Series Bull. No. 94, 1964).

Drying of the Sample

Young et al., (1953) placed the diet composites in a shallow drying tray in a freezer for twelve hours. The sample was then placed in a Desivac for drying, then weighed and ground. Analyses of fat and nitrogen were performed on the dry samples.

In the study conducted by Kaucher and co-workers (1945) the homogenized samples were dried to a constant weight at 60°C in an oven. When constant weight was achieved, the samples were weighed, ground, and then stored in a brown bottle in a dessicator. They determined vitamins, fat, protein, and energy on these samples.

Bassett and Van Alstine (1935) in preparing their diets for nitrogen, sodium, potassium, magnesium, calcium, phosphorus, chlorine, and iron analysis dried the foods at 90° to 95° C. The sample was then pulverized and stored.

Drying at a temperature of 70° C and higher according to Teague et al., (1942) may result in volatilization of essential oils, loss of

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nitrogen and decomposition of fats and carbohydrates. They further report that a protein containing substance after having been dried to a constant weight may still contain 2 to 3 per cent water.

III. Two Methods For Determining Food Composition

The energy and nutrient content of a diet can be determined by two distinctly different methods: (1) laboratory analysis of either the individual food items of a diet or the prepared aliquots of the total diet, or (2) calculations from tables of food composition. The first concerns controlled experimental investigations, while the latter has the potential for adaptation to field epidemiological studies (Leverton et al., 1960).

Leverton and co-workers (1960) reported that the calculated food values would only present a range of that nutrient in which the food, when analyzed, will fall. Thus food values obtained by the two methods should not agree exactly. The figures in the tables are derived in different ways and provide somewhat different information about the product consumed. The calculated values as emphasized by Leverton et al., (1960) provide averages of the nutritive values of the diet or similar foods on a year-round basis.

Widdowson and McCance (1943) stated that composition tables present the organic constituents in a food for comparison with other foods. They do not give any information as to the exact amount of nutrients which a particular item contains. Harris (1962) stated that food tables do not present data with an accuracy of atomic weight determinations, neither are they so unreliable as to be worthless.

Food Composition Tables

The constituents of each food as listed in the tables of food composition represent the average values derived from chemical analysis. The number of values which composes the mean varies for each nutrient. Patterson and McHenry (1941) reported that occasionally only minimal or maximal figures are presented. Leverton et al., (1960) postulated that if there is a vast amount of data for a particular item, the average of the values can be considered fairly reliable, whereas, those with the least number of values will be more in error when related to the analyzed values. The term year-round is considered in tabulating the data for the tables. This takes into account variety, seasonal, and geographical differences, the consequence of storage for periods prior to marketing,and the effect of manufacturing and preparation practices (Leverton et al., 1960). A factor not mentioned by Leverton et al., (1960) is the error inherent in analytical techniques. Thomas and co-workers (1950) pointed out that the analytical values do include variability which is attributable to sampling, preservation, and measuring as well as errors inherent in the analytical methodology.

Conversion Factors

Errors may also be prevalent in food tables because of the conversion factors employed. The determination of conversion factors for the calculation of food energy from carbohydrate, fat, and protein and those for computing protein from the determined nitrogen have been greatly neglected (Mayer, 1952).

Calories

A distinction is required between the calories as derived from the heat of combustion and those which represent the physiological value.

The former is the number of calories produced by burning a weighed amount of food in a bomb calorimeter. The latter represents the food energy that is available for the body. This value takes into consideration the digestability coefficient and the energy lost to the body through urea formation.

Atwater (1899) compiled from different groups of people residing in the different areas of the U. S. one hundred eighty-five dietaries. He calculated the per cent of protein, fat and carbohydrate in the diets for the following food groups: meats, milk, cereals, sugar, vegetables and fruits. Heat of combustion values were then determined for the protein, fat and carbohydrate in each food group (See page 43 for values). The physiological value was derived by multiplying the digestion coefficient, which was determined for each division of foods, by the amount of protein, fat and carbohydrate contributed by the separate groups. For protein 1.25 calories per gram were substracted for the nitrogen compounds excreted in the urine. These values were then adjusted according to the extent that each food group occurred in the U. S. diet at that time (Maynard, 1944). The values thus secured are: 4, 9, 4 calories per gram of protein, fat and carbohydrate, respectively

The Committee on Calorie Conversion Factors and Food Composition Tables (1947) found no evidence that Atwater's values, if properly used, were not reliable. However, if the proportions of food are not similar to his averaged mixed diets, the application of these values can present inaccurate food energy values (Maynard, 1944). Mayer (1952) stated that if the common figures are used in computing the physiological value of a high bulk, low calorie diet the calculated values would be

too high. It is emphasized by Maynard (1944) that Atwater never intended that the factors 4, 9, 4 should be applied without modification to all diets. For mixed diets which deviate from the average U. S. diet the caloric values may be determined by two methods. They are: (1) applying the individual fuel values for protein, fat and carbohydrate, respectively, to each food or food group and (2) readjusting the proportions in the average mixed diet which is 60:40, animal:vegetable, and determining new average values for the protein, fat and carbohydrate components, respectively.

If the caloric value of a diet as determined by a bomb calorimeter is to be compared with the calculated values, the values that Atwater employed in calculating the conversion factors 4, 9, 4 should be used.

Protein

Protein in a food is generally calculated from its nitrogen content which is then multiplied by a conversion factor. These are the values in food composition tables. Some food composition tables assume: (1) all nitrogen in the food is protein and (2) all proteins contain 16 per cent nitrogen (Mayer, 1952). Jones (1931) reported that there are few nitrogenous substances whose nitrogen is only in the form of protein. Because of these gross assumptions the Committee on Calorie Conversion Factors and Food Composition Tables (1947) classified the common nitrogenous foodstuffs into five groups and determined the conversion factors for each division. They are: rice, 5.95; refined wheat, 5.70; oilseeds and nuts, 5.30; meat and eggs, 6.25; milk, 6.38; and fruits and vegetables,6.25. The latter factor for fruits and vegetables is employed since there are no data to justify calculating a special factor. If food composition tables determine protein values by

using the conversion factor 6.25, significant errors could result when the research involves the comparison of the calculated and the analyzed values.

There are many tables of food composition available. At best each of them presents only approximate values for the nutrient content of foods. According to Mayer (1952) there are two documents which take into consideration the specific conversion factors as mentioned previously for estimating calories and protein. The value presented for calories in these tables is the physiological value. These two are the Food and Agriculture Organization (United Nations) Food Composition Table for International Use (Chatfield, 1949) and Agriculture Handbook No. 8 of the United States Department of Agriculture Composition of Foods--Raw, Processed, Prepared (Watt and Merrill, 1963). Mayer (1952) stated that the U.S.D.A. publication may be considered as the authoritative document for American foods.

Calculation versus Analysis

The constituents to be discussed in this comparison are protein, fat, and food energy. The analytical methods employed for the chemical analysis are as follows: protein, Kjeldahl; fat, ether extraction; energy, bomb calorimeter.

Protein and Nitrogen

None of the investigators presented in the following section indicated the conversion factors which were employed to compute the grams of nitrogen from the calculated protein value or the amount of protein from the analyzed nitrogen. These computations are necessary in order to compare the calculated with the analyzed values for protein and nitrogen. By implication it is possible to assume that the values used

were the same as those presented in the various tables of food composition employed to calculate the diets.

Carroll et al., (1952) compared the calculated and analyzed protein values for six diets from four cottages of a children's camp. Analysis of variance revealed no significant differences between the two sets of values. However, even though not significant the chemical analyses resulted in higher values than the calculated in three of the four cottages.

Wertz et al., (1952) found that the calculated values for protein of twenty-one diets did not differ significantly from the determined values. The statistical measure employed was the "t" test.

The nitrogen in the foods served in a hospital metabolic ward was computed and analyzed by Bassett and co-workers (1931). They found close agreement between the two methods. The statistical procedures used to verify the authors' suggested close agreement were not reported.

Lutwak et al., (1964) in conducting a metabolic balance study on children investigated the two methods for nitrogen. The authors report an agreement that was within 10 per cent. Widdowson and McCance (1943) found similar results.

Bransby et al., (1948) disagree with the preceding. They found a statistically significant difference between the two sets of values for protein. The statistics employed were not discussed. The average analyzed value of the thirty-three composite diets was 76 grams of protein per day, whereas, the average calculated value was 68 grams. The composite represented the diets from three days of individuals living at home. The standard deviation for the absolute difference in the thirty-three composites was 5.6. Sixteen of the calculated values for

the diets revealed a difference of greater than 10 per cent of the analyzed.

A consistent difference, even if slight, between the calculated and analyzed values for nitrogen might be attributed to a variation in the composition of foodstuffs produced under differing conditions of atmosphere and soils. Donelson et al., (1931) reported the mean determined value of nitrogen was 1.9 per cent higher than the calculated. They encountered, on isolated days, marked divergences between the calculated and determined values, whereas, on other days the two were very close.

Fat

Diets of six subjects in which exact duplicates of the food eaten were saved for analysis revealed that in five out of the six, the calculated fat values were higher than the analyzed amounts. Two of the six calculated values exceeded the analyzed values by more than 10 per cent.

Wertz et al., (1952) reported that the calculated values for fat were significantly higher than the analyzed values. Of the twenty-one diets eleven of the calculated values fell within 5 per cent of the analyzed values. Significance was measured by the "t" test.

The calculated fat values of three moderate fat diets correlated closely with the analyzed values (Wollaeger et al., 1947). The analyzed fat was 100.5 grams, whereas, the calculated value was 101.6. Lutwak and co-workers (1946) found similar agreement. The analyzed values for fat in their diets were within 10 per cent of the calculated values.

Fat and Calories

Fat and calories were determined by calculating and analyzing the thirty-three composite diets in the studies conducted by Bransby

et al., (1948). The absolute and percentage difference between the average nutrient values for all diets were not statistically significant for calories or fat. For the individual diets the calculated values were within 10 per cent of the analyzed in eighteen and twenty-seven of the thirty-three composites for fat and calories, respectively. They suggest that the better agreement for calories is due to the fact that an over-estimate of the energy as derived from one of the three sources of food energy, carbohydrate, protein and fat, is counterbalanced by an underestimate of the other.

Protein, Fat and Calories

McCay et al., (1945) conducted studies to evaluate the nutritive value of the diets consumed at four large naval training stations. They compared the values calculated for nitrogen, calories and fat with the determined results. They reported that the calculated values were a fair approximation of the determined values for nitrogen and calories, however, the calculated value of fat was greater than the analyzed amount. According to the authors the fat differences were probably due to an over estimate of the amount of fat per cooked serving of meat. It is difficult to understand why they reported that the analyzed food energy is a fair approximation of the calculated value when the calculated values for both diets were greater than the analyzed values by more than 10 percent.

Similar trends are reported for thirty diets consumed by twelve lactating women (Kaucher et al., 1946). The calculated value for fat was fourteen per cent higher than the analyzed amount. The calculated values for protein and calories were within 1.7 and 0.6 per cent, respectively, of the analyzed values.

The authors reported that their analytical data represented food which had been subjected to the hazards of marketing, storage, preparation, cooking and serving, while the calculated values represented the fresh foods.

Patterson and McHenry (1941) reported that for calories the calculated values are very close to the determined values for the same diet. In the case of protein the calculated average is slightly lower, but is still in good agreement. Their results are based on twenty different lunches. The comparison of the two methods is reversed for fat, and the large discrepancy leads to the opinion that the values for fat in the food tables may be too great or that the loss of fat during food preparation is fairly large. In their studies the deviation of individual values from the mean is significant for protein and even larger for fat. Such wide deviation indicates that the carbohydrate value for foods, as listed in the food composition tables, is too low. This assumes that the carbohydrate value was obtained by difference.

Leverton and Whiting (1960) collected approximately three hundred cases in the literature which compared the two methods of determining the nutritive composition of a food. The studies were performed in the United States, Great Britain, and Canada. For protein and calories the calculated values for more than 50 per cent of the cases fell within a range of 10 per cent of the analyzed values. The calculated values for fat were considerably higher than the analyzed determinations. In fact, this factor was so consistent that if a chemically determined value for fat was 140 grams then the calculated figure ranged from 130 to 185 grams. They concluded from their study that the data available in in the literature support the fact that for epidemiological investigations the validity of food tables for giving a range of the actual intake is questionable.

Individual Items versus Total Diet

Theoretically, the results of analyses of individual dietary components should, on addition, provide the same value as that secured from analysis of the total diet. To explore this possibility Hummel et al., (1942) analyzed twenty-two foods individually and as a composite diet. These foods represented the foods consumed by normal children over a period of several years. The results obtained from the individual analysis of milk and banana more closely approached the values recorded in food tables than for any other food analyzed. They reported that the variability from time to time of the same foodstuffs is due to changes in composition. The fat content of the analyzed foods showed the greatest variation from the calculated figures. The individual values for the same diet were within one standard deviation from the mean of the analysis of the dietary composites for fat, nitrogen and food energy. The composite diets showed a more constant composition than the components. There was also better agreement between the analyzed and calculated values for the composite than when the sums of the analysis of the individual foods were compared with the calculated values.

Hunter and co-workers (1948) stated that the greater the number of different food items in the diet being analyzed, the greater the chances that the computed nutritive value will be correct. This statement would not apply to an unstable nutrient like ascorbic acid. If the analysis was not performed promptly on such diets or adequate means adopted to prevent the destruction of the nutrient prior to analysis, regardless of the number of food items, the analyzed results would not approach the calculated values.

Individual Versus Average Diet Analysis

Toscani (1948) compared the analyzed and calculated values for nitrogen in twelve diets. The diets were analyzed individually and then as a composite. The individual diet analyses showed a large variation when compared to the calculated nitrogen value. These analyzed values ranged from 0.0 to 9.6 per cent below the calculated values. The analyzed averages were only 3.7 per cent below the calculated value.

Meyer and co-workers (1951) compared the analyzed values for the nutrients in school lunches with the calculated values. Single lunches were collected as well as the composites of the five lunches. There was a wide range of difference between the calculated and analyzed values for the single meals. However, for the composites the calculated values were within 5 per cent of the analyzed values for calories, protein and fat. Bransby et al., (1948) upon averaging the diet composites of thirty-three subjects in their study found close agreement for protein and calories. However, the values for the individual diets were so diffuse that it was extremely doubtful, according to the authors, as to whether it was worth the time and effort to calculate the individual diets. The difference between the calculated and analyzed values for calories was within 10 per cent in 82 per cent of the diets. For fat and protein nearly 50 per cent of the analyzed values for the diets were not within 10 per cent of the calculated values.

Variation in Analyses of Identical Diets

Hawks and co-workers (1937) hypothesized that there are variations in the composition of identical diets when when experimental conditions are carefully controlled. They analyzed eight and eighteen identical

diets, respectively, in two experiments for nitrogen and calories. The correlation coefficient of variation ranged from 1.9 to 2.7 per cent for calories. For nitrogen the spread was 1.6 to 3.0 per cent. The coefficient of variation represents the extent to which the standard deviation will vary from the mean. They presented three causes for these variations in the composition of the same diet: (1) unavoidable errors in chemical technique, (2) difficulty in obtaining a homogeneous sample of food for analysis even under strictly controlled conditions and (3) variation in the composition of food.

Bassett et al., (1931) prepared and analyzed the same diets for nitrogen in different years and different seasons. Their average analytical results agreed closely with the calculated values of all the diets. They postulated that if a researcher throughout the course of a study averaged the values obtained from analyzing a diet which was replicated at ten times during the study the result would be the actual composition of five of the ten diets.

Reifenstein et al., (1945) in comparing the same diet prepared at two different times and another diet prepared at three intervals found a consistent 11 per cent deviation for the analyzed nitrogen values of the same diet. Bassett and Van Alstine (1935) pointed out that the fluctuations in the analyzed value for nitrogen of the same diets represent the inherent variations in the concentration of the constituents of the same food from time to time.

Differences in Analysis of Duplicate Samples

Hawks et al., (1937) reported that duplicate diets collected on the same day and in the same manner varied as much as 4.8 per cent while

the average differences ranged from 1.0 to 2.0 per cent, respectively, for calories and nitrogen. Again the variation in the composition of food must be considered in these results. The differences observed in the duplicate samples were less than those recorded for the same diet analyzed on different days.

Upon analyzing duplicate samples, Thomas et al., (1950) found the values for protein and food energy to be the most consistent and showed little variation. However, the determined fat in the duplicate samples varied immensely. These inconsistent differences, as hypothesized by the researchers, are due to sampling. Many of the diets used in this study contained mixed dishes. These were prepared in large receptacles. It is impossible to control with any precision the amounts of the individual constituents of the dishes prepared in this manner. The only way that a mixed dish can be prepared in a more exact duplication is that the ingredients for each serving be weighed, cooked and served in the same utensil. The authors concluded that this procedure is costly, yet in highly controlled studies it is more efficient to use food preparation more amenable to exact sampling.

It is the purpose of this study to explain the dietary procedures appropriate for a non-clinical metabolic balance study, to discuss the calculated and analyzed values of the same diet, to present the variability of composition of the same diet from different weeks of the study, and to determine the time a sample should be removed for analysis to assure that it represented the diet of the subjects consumed.

EXPERIMENTAL PROCEDURE

A metabolic balance study was devised to investigate bread as a primary source of protein for man. The subjects were twelve male college students. They were selected from forty volunteers who were obtained by notifying several campus departments and through verbal contact. The volunteers met with Dr. Olaf Mickelsen and the two graduate students assisting with the study. At this time the purpose of the study and the subject's responsibilities were explained. The potential subjects were asked to record their age, height, weight, religion and food likes and dislikes. Only those who were within 10 per cent of their weight as designated by standard height-weight tables were chosen. Those subjects who were married or who did not live close to the campus were eliminated. The subjects with food dislikes which were not coherent with the objective of the study were rejected. As a check on the subject's honesty, responsibility and adaptability to the experimental controls, they were given the Minnesota Multiphasic Personality Inventory Examination. The results of this and the assessment of their health status by a medical examination served as the final basis for the selection of the subjects. The subjects chosen were three foreign and nine American students. Their ages ranged from nineteen to twenty-seven years.

Each subject at the beginning of the study was given a notebook in which he was to record daily activities, the amount consumed of the ad libitum items which were sucrose, tea, coffee and hard candies, and the amount of daily rest. Any abnormal physical conditions were also to be recorded. Also at this time each subject was assigned a number.

The first part of the study consisted of twenty-one days during which the subjects consumed the control diet. This was immediately followed by a fifty day experimental diet. One week of the control regimen followed the experimental period. The prime objective of the study was for the diets to contain the same amount of protein in both the control and experimental regimens.

Animal protein in small amounts was served during the control period, whereas, throughout the experimental phase no animal protein was allowed. The control regimen was calculated to supply an average of 68.3 grams of protein, 115.6 grams of fat and 3,086 calories per day (see Table 1). The diets (Table 2) contained only small amounts of milk, eggs, meat and other animal protein. The experimental regimen was calculated to provide an average of 63.6 and 89.2 grams of protein and fat, respectively (Table 1). Wheat products accounted for 70 per cent of the calories and 90 to 95 per cent of the protein. The remaining protein came from fruits and vegetables (Table 3).

The results obtained from the subject during the control diet were compared with the results of the same subject on the experimental regimen.

The food was prepared by two cooks in the metabolic kitchen in the Home Economics Building. One cook worked between the hours of 5:30 a.m. and noon. The other from noon to 6:30 p.m. Two students were employed on a part-time basis to wash dishes and to clean the kitchen and the dining areas.

All meals with the exception of the Sunday morning and Sunday evening meals were served in the Home Economics Building. The Sunday breakfast and supper were sack lunches. This was for economical reasons and because of the subjects request for free Sunday mornings

and evenings. Only one cook was required to prepare Sunday's meals. Meals were planned which could be packed and yet would provide the same meticulous measurement as if the subjects received their meals directly from the kitchen (Control Diet VII, Table 4 and Experimental Diet VII, Table 5). The sweet rolls for Sunday breakfast during both periods were baked on Saturday. The orange and sweet roll and the bread, in the case of the experimental diet, were weighed and packaged in cellophane sandwich bags. These were placed in paper sacks which were labeled with the subject's number and were presented to the subjects Saturday evening. The Sunday evening meal which consisted of sandwiches, fresh fruit, and a baked dessert were weighed Sunday morning, wrapped as previously described and given to the subjects Sunday noon. On Monday through Saturday the subjects were given, at dinner, a snack of either cookies, bread or crackers which was to be consumed that evening (Tables 4 and 5). The snacks were packaged in sandwich bags and labeled with the subject's number.

The control and experimental diets referred to the structure of the diet designed for each period of the study. When used with the Roman numerals, they designated a particular day of each regimen. Roman numeral I through VII were the diets served Monday through Sunday, respectively. The three meals, complements and snack composed one day's menu or diet (Tables 4 and 5).

A seven day menu cycle was designed for each period (Tables 4 and 5). The carbohydrate, protein and fat content of the menus and the recipes (appendix) were calculated with the aid of the U.S.D.A. Handbook No. 8 (Watt and Merrill, 1950 and 1963) and Bowes and Church (1963).

All recipes required by the menus were calculated on the following basis. The ingredients were listed with the amount required in grams. The carbohydrate, protein and fat in each ingredient were computed from the food tables and totaled. If the product demanded a mode of cookery, the weight prior to serving was determined. This weight was obtained when the recipes were standardized for time and temperature two months before the study began. The grams of carbohydrate, protein and fat per serving were then calculated in proportion to the prepared weight of the recipe. Please refer to the appendix.

All foods employed in the study were purchased from the Michigan State University Food Stores, Michigan State University Dairy Store and a local grocery store. Whey-free butter was used for the experimental phase of the study. It and the butter were produced by the dairy department on the campus.

The frozen food items were purchased at the beginning of the study in sufficient quantity for the entire study. The dry storage items, such as flour and sugar, that could not be stored for the duration of the study due to inadequate storage facilities were ordered at the beginning of the study and stored until required at the University Food Stores. The perishable items such as fresh fruits and vegetables were purchased twice a week and were of the same variety. The meat and the eggs for the control period were purchased in sufficient quantity for the length of this regimen. The meat was packaged in the amount required by each day's menu. It was of the same variety and cut.

The fruits and vegetables used were frozen with the exception of those listed as canned (Tables 2 and 3) or fresh. Apples, bananas and oranges were the fresh fruits. The fresh vegetables were lettuce

radishes, green peppers, cucumbers, cabbage, tomatoes, celery and carrots. All juices used were frozen and diluted with water according to the specified directions on the container. The frozen strawberries were sliced and sweetened.

The ingredients for each recipe and the food items served were weighed on a Hanson spring scale and a Toledo scale. The Hanson scale was employed for food items such as butter, jelly, sugar, salads, meats, bread, crackers, and desserts; whereas, the Toledo scale was used for weighing the ingredients of the mixed dishes and the foods served other than those mentioned previously.

Upon weighing, the ingredients for the mixed dishes were mixed thoroughly. In the preparation of the desserts and the bread, the ingredients were mixed with the aid of an electric mixer. After the mixed dishes were prepared, the amount specified per serving was weighed into individual casseroles. The filled casserole dishes were then covered and placed in a warming oven until they were served to the subjects. The only mixed dishes not treated in this manner were the dumpling and vegetarian stew in diet I (Table 3) and the pizza dough and sauce in diet VI (Table 3). The dumplings and the vegetarian stew were cooked in a large receptacle and the individual servings were weighed when the subjects arrived to be served. For the preparation of the pizza, dough was weighed as wet dough per individual serving and a designated amount of pizza sauce was placed on each individual pizza tray.

For the preparation of the meats served during the control regimen, please refer to the appendix.

The bread and all desserts in the menus were prepared daily. The

French bread, hot rolls and sweet rolls were made from the bread recipe (appendix p.126). The amount of bread required for each meal was weighed and packaged in cellophane bags prior to serving.

The salads, fruits, and desserts were weighed and covered with saran wrap prior to the serving periods.

The butter, jelly, honey and sugar were weighed in containers labeled with the subject's number.

The method of cookery used for the hot cereals is listed in the appendix under each specific cereal.

The diets supplied the recommended nutritive allowances with the exception of calcium. Calcium was sufficient in the control diets, however, during the experimental regimen the diets supplied an average of 250 milligrams of calcium per day. In order to compensate for this deficiency, calcium lactate pills which supplied 650 milligrams of calcium were given to the subjects daily.

The energy supplied by the diets was calculated by two methods. The average physiological values of 4, 9, 4, for carbohydrate, fat and protein, respectively, as derived by Atwater (1899) and the specific heat of combustion factors for the particular groups of food. These are the following:

Food	Carbohydrate	Protein	Fat
	(calories per gram)		
milk	3.95	5.65	9.25
meat, fish	3.95	5.65	9.50
eggs	3.95	5.75	9.50
fruit	4.00	5.20	9.30
vegetables	4.20	5.00	9.30

Food	Carbohydrate	Protein	Fat
	(Calories per gram)		
wheat and cereals	4.20	5.80	9.30
butter fat	--	--	9.25
animal fat	--	--	9.50
vegetable fat	--	--	9.30

The conversion factors employed to determine the amount of nitrogen in the diet from the calculated protein were those of Jones (1931). These factors for the respective food groups were milk, 6.38; meat, eggs, fish, 6.25; fruits and vegetables, 6.25; wheat and cereals, 5.70; and nuts and seeds, 5.30. The amount of nitrogen supplied by each food group was determined by dividing the protein content of a food group by its specific conversion factor.

Since the objective of the study was to determine the effect of a wheat diet on the nitrogen metabolic pattern in the subjects and, furthermore, because weight loss or gain affects nitrogen balance, it was most important to maintain the subjects' weight. During the control period there was no change in weight (Bolourchi, personal communication 1965). However, during the experimental regimen some of the participants began either to loose or to gain weight. Additional calories were supplied to those subjects losing weight in the form of whey-free butter, jelly, hard candies, honey and sugar. These foods were allowed ad libitum; however, the amount consumed was weighed and recorded in the kitchen. For the subjects who were gaining weight, dietetic jelly was allowed. Their whey-free butter and sugar intakes were rationed. They received no hard candy or honey.

The items allowed ad libitum to the subjects were coffee, tea, salt and sugar. The sugar was not allowed ad libitum to those subjects who were gaining weight. For this purpose instant coffee, tea bags and sugar packs were employed. All items issued to the subjects were recorded. The subjects were requested to record in their record books the teaspoons of coffee, the number of tea bags and sugar packs that were consumed each day. The powdered coffee and tea were analyzed for nitrogen. These results were employed to tabulate the additional amount of nitrogen consumed by each subject.

A trial period utilizing five of the experimental menus was employed prior to the balance study. The participants in the trial period were not the same subjects who were chosen for the study. Both males and females were in the trial group. The food was prepared and served in the metabolic kitchen of the Home Economics Building. Food samples which represented the same amount the subjects were to receive were collected for analyses each day. The preliminary study occurred one week prior to the actual study and was five days in length.

Because the balance period for the study was not the same length as the number of menus, the diets were analyzed each day. The determined values were totaled to represent each balance period. The length of the balance period was five days.

During three weeks of the experimental period food samples of the same amounts as served on the menus (Tables 4 and 5) were removed for analyses when the first subject was served at zero hour. During the fourth week of the experimental regimen the components of two diets were collected. The first diet was removed as previously stated. The second diet was removed when the last subject was served at one and

one-half hour after the serving time had begun.

To further evaluate the time a diet should be removed for analyses to be assured of the subject's intake, two diets were prepared. The meals of the diets were collected at zero, one-half, one, and one and one-half hours after the serving time had begun. Individual food items of three diets were collected separately for analyses. These samples were weighed into Sealtest 16 ounce plastic-lined cartons. The total diets for the same three days were also collected.

Upon collection the food items were placed in a tared #10 tin can. The weight of the container was recorded on the can with a magic marker pencil. The container was covered with a polyethylene cover and placed in the freezer after the addition of each item. At the end of each day the container plus the contents which included the three meals, complements and snack were weighed. The weight was recorded on the container as well as the date and the diet number. This was stored overnight in the freezer. The contents were then transferred with the aid of 710 ml. of distilled water and a rubber spatula into a Waring blendor. The diets were homogenized for five minutes at medium speed and five minutes at high speed. Upon homogenization the slurries were put into 16 oz. Sealtest plastic-lined cartons. The remainder of the slurry was discarded. The cartons were labeled with the date and the diet number and placed in the freezer until the analyses could be performed.

The wet slurries when removed from the freezer were thawed for twelve hours. Then approximately 16 grams of the wet diet were placed in a tared evaporating dish. All analytical weighings were performed on a Mettler Balance. They were dried to constant weight at 60°C in an oven with a vacuum of twenty-eight inches of Hg. Upon drying they

were ground in a Wiley Mill using a #20 mesh screen. The ground, dried samples were placed in labeled bottles which were covered with plastic screw tops. These were then placed in a dessicator.

The per cent moisture was determined by the difference in weight between the wet sample and the dried sample. The actual per cent moisture per diet was computed with the aid of the following formula:

(1) Wet weight of collected diet + water added x (100 - avg. % moisture) = dry matter.

(2) (Dry matter/gm. wet diet) x 100 = % dry matter.

(3) 100 - % dry matter = % actual moisture in original diet

The moisture was determined on the samples at two times. The time intervals were Spring, 1964 and Spring, 1965. In 1964 the samples were only dried for twenty-four hours at 60°C in an oven using a vacuum of twenty-eight inches of Hg. In 1965 the samples were dried as previously described.

One gram dried samples were weighed in duplicate for nitrogen determination by the macro-Kjeldahl method. The formula used to calculate the amount of nitrogen per sample was:

$$\text{gm. of N in sample} = \text{ml. HCl (titration-blank} \times \underline{N} \text{ of HCl} \times 0.014 \text{ [milliequiv. wt. of N]}$$

$$\% \text{ N (dry wt. basis)} = \frac{\text{gm. N/sample}}{\text{sample wt.}}$$

$$\text{gm. of N/diet} = \% \text{ N} \times \text{dry wt. of diet}$$

For fat determination duplicate 1 gram dried samples were extracted with ethyl ether for three hours in the Goldfisch apparatus. The ether was evaporated and then the beaker which contained the fat was weighed. The weight of the dry beaker substracted from the former value was the amount of fat per sample. The grams of fat in each diet were calculated with the aid of the following formula:

$$\text{gm. of Fat in diet} = \frac{\text{wt. fat extracted}}{\text{wt. dried sample analyzed}} \times \text{gm. dry diet}$$

One gram pellets were formed in duplicate from each dried diet sample for the energy determinations. The instrument employed to measure calories was the Parr Oxygen Bomb Calorimeter. Correction factors were employed for the nitric acid produced and the unburned fuse wire (Parr Instrument Company, 1960). The nitric acid was titrated with 0.0508 N NaOH. One ml of NaOH is equivalent to 0.801 calories and one cm of fuse wire is equivalent to 2.3 calories. The formula employed to calculate the heat produced in calories per gm of dry sample was:

$$\text{Gross heat in calories/gm} = \frac{(t)\ (w) - c}{S}$$

t = temperature increase

w = water equivalent of the calorimeter = 1364 cal/degree F

c = calories of total correction from unburned wire and acid formed

s = sample weight

The water equivalent was determined by combusting standard benezoic acid.

Upon analyses, the determined values were compared with the calculated values by the difference as per cent analyzed, standard deviation, 3-way analysis of variance and the "t" test.

The standard deviation was determined with the aid of the formula given in Dixon and Massey (1957).

$$\text{s.d.} = \left[\sum_{i=1}^{N} (x_i - \bar{x})^2 / (N - 1)\right]^{\frac{1}{2}}$$

where N = the number of observations

x_i = the value of each observation

$\bar{x}$ = the mean of the observations.

A three-way analysis of variance was used to determine significance between the calculated and analyzed values for the same diets. This also determined the significance between the calculated and analyzed diets of one week of the experimental regimen when compared with the other weeks and the significance between the calculated and analyzed diets within the same week. The calculated "F" values were compared with those listed in the "F" distribution tables in Dixon and Massey (1957).

The "t" test was employed to further determine the reliability of the food tables and differences in sampling time.

$$t = (M_1 - M_2) / \quad (S.E.M._1^2 + S.E.M._2^2)^{\frac{1}{2}}$$

Where M_1 = the mean of one set of observations

M_2 = the mean of the second set of observations

SEM= Standard error of the mean of each observation

The standard error of the mean was determined by the method of Mantel (1951). By this method the standard error is the range of values for a set of observations divided by the number of observations. This method is very accurate for samples of fifteen or less (Mantel, 1951). The "t" test was also used to determine the probability that the analyzed values agreed with the values calculated from the food tables.

One-way analysis of variance was applied to determine the significance between the same diet replicated at four intervals during the experimental period. The calculated "F" values were compared with those listed in the "F" distribution tables in Dixon and Massey (1957, p. 388).

In order to evaluate the meals collected at the four time invervals the maximum deviation was expressed as per cent of the mean.

Table 1

Composition of the Diets[1]

Diet	Control				Experimental			
	CHO gm.	Protein gm.	Fat gm.	Energy[2] Cal.	CHO gm.	Protein gm.	Fat gm.	Energy[2] Cal.
I	394.2	66.4	111.7	3052	500.9	65.4	84.8	3258
II	373.1	71.4	131.5	3184	469.8	64.7	86.4	3142
III	382.5	66.7	121.1	3095	477.8	61.8	87.3	3154
IV	409.4	67.7	113.9	3150	513.3	64.4	95.8	3408
V	382.6	68.6	110.4	2999	481.7	62.4	80.7	3121
VI	403.7	68.2	109.2	3082	483.7	62.4	94.4	3253
VII	388.3	69.2	111.7	3043	471.7	64.0	95.4	3228
Mean	390.6	68.3	115.6	3086	485.5	63.6	89.2	3237

[1] The composition of the diets represent the calculated values obtained from tables of food composition.

[2] The Atwater (1899) heat of combustion factors were employed in computing the food energy.

Table 2

The composition of the foods comprising the control diets [1]

Diet I					Diet II				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Grapefruit Juice	150	15.6	0.7	0.2	Orange Juice	150	16.3	1.2	--
Jelly	20	13.0	--	--	Jelly	20	13.0	--	--
Carrot Sticks	25	2.3	0.3	0.1	Onion Soup(canned)	200	3.5	4.4	2.1
Celery Sticks	25	0.9	0.3	0.1	Lettuce	50	1.4	0.6	0.1
Lettuce Wedge	50	1.4	0.6	0.1	Tomato	50	2.0	0.5	0.1
Potatoes	100	19.1	2.0	0.1	Carrots (canned)	75	4.8	0.5	0.4
Green Beans (canned)	75	3.5	1.1	0.1	Shredded Cabbage/ Pineapple Salad	50	2.7	0.7	0.1
Lime Sherbet	75	22.6	1.2	1.1	Milk	240	11.8	8.4	9.4
Milk	240	11.8	8.4	9.4	Butter	50	--	0.5	40.5
Butter	45	--	0.5	36.4	Pork Chop	60	--	13.6	15.5
Spanish Rice	300	50.1	6.3	7.0	Oatmeal	236	31.2	6.5	3.4
Roast Beef	50	--	15.6	5.8	Bread	200	105.2	17.9	7.1
Puffed Rice	14	12.3	0.8	3.0	Bread Dressing	150	26.3	4.6	8.2
Bread	200	105.2	17.9	7.1	Saltine Crackers[2]	25	18.0	2.2	3.1
Peach Crumb Pie	135	68.0	3.4	12.6	Potato Chips	20	9.3	1.3	7.0
Ginger Cookies	50	33.5	3.1	7.7	Cherry Kuchen	100	44.8	3.3	12.4
Refrigerator Cookies	60	34.9	4.2	20.9	Applesauce Cake	75	44.9	2.7	12.1
		394.2	66.4	111.7	Crunchy Cookies	50	35.9	2.5	10.0
							373.1	71.4	131.5

Table 2 (Cont.)

DIET III					Diet IV				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Grapefruit Juice	185	19.2	0.9	0.2	Orange Juice	150	16.3	1.2	--
Apricots (canned)	120	21.4	0.6	0.1	Melon Balls	100	15.7	0.6	0.1
Jelly	20	13.0	--	--	Jelly	20	13.0	--	--
Green Pepper	20	1.2	0.2	--	Lettuce	20	0.6	0.2	--
Lettuce	30	0.9	0.4	0.1	Green Pepper	10	0.6	0.1	--
Tomatoes (canned)	100	3.9	1.0	0.2	Tomato	20	0.8	0.2	0.1
Rice	100	26.2	2.5	0.1	Carrot/Pineapple Salad	50	7.0	0.4	0.1
French Fries	50	26.0	2.7	9.6	Potatoes au Gratin	130	18.2	4.0	5.2
Dill Pickles	30	0.6	0.2	0.1	Asparagus	50	1.8	1.2	0.1
Catsup	15	3.7	0.3	0.1	Harvard Beets	100	24.0	0.7	3.7
Mustard	10	0.6	0.5	0.4	Milk	240	11.8	8.4	9.4
Milk	240	11.8	8.4	9.4	Butter	40	--	0.4	32.4
Butter	50	--	0.5	40.5	Canadian Bacon	50	--	14.8	10.2
Beef Patty	40	--	10.3	7.2	Sweet Roll	130	70.1	9.5	15.3
Roast Chicken	40	--	11.3	3.4	Rice Krispies	28	25.1	1.6	0.1
Sh. Wheat Biscuit	22	18.3	2.2	0.3	Bread	170	89.4	14.2	6.0
Bread	100	52.6	8.9	3.5	Chocolate Cake	100	55.6	3.8	17.3
Hamburger Bun	38	20.9	3.4	2.1	Molasses Cookies	40	25.9	2.3	6.2
Cupcake	50	31.6	3.5	7.2	Ginger Cookies	50	33.5	3.1	7.7
White Icing	30	24.5	--	4.5			409.4	67.7	113.9
Lemon Pie/Meringue	160	77.0	5.4	14.7					
Refrigerator Cookies	50	29.1	3.5	17.4					
		382.5	66.7	121.1					

Table 2 (Cont.)

Diet V					Diet VI				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Banana	100	23.0	1.2	0.2	Grapefruit Sections (canned)	100	18.5	0.5	0.2
Apple	230	25.3	0.5	0.2	Pears (canned)	115	21.1	0.2	0.1
Jelly	50	32.5	--	--	Fruit Salad	50	8.2	0.3	0.1
Vegetable Soup(canned)	200	10.5	2.6	1.3	Lemon Sauce	50	15.8	0.2	3.2
Celery Sticks	25	1.8	0.6	0.1	Jelly	30	19.5	--	--
Lettuce	50	1.4	0.6	0.1	Lettuce	20	0.6	0.2	--
Peas	50	6.4	2.7	0.2	Fr. Cut Green Beans	100	6.1	1.7	0.1
Peanut Butter	20	4.2	5.2	9.6	Potatoes	75	14.3	1.5	0.1
Mashed Potatoes	100	18.3	2.1	2.4	Milk	240	11.8	8.4	9.4
Milk	240	11.8	8.4	9.4	Butter	50	--	0.5	40.5
Butter	45	--	0.4	36.4	Creole Spaghetti Sauce	100	4.7	3.4	4.5
White Fish (raw wt.)	60	--	13.8	3.8	Veal Cutlet	40	--	13.3	6.1
Corn Flakes	25	21.0	2.1	0.1	Spaghetti	100	30.2	5.1	0.6
Bread	200	105.2	17.9	7.1	Puffed Wheat	12	9.5	1.6	0.2
Saltine Crackers[2]	25	18.0	2.2	3.1	Bread	225	118.3	20.1	7.9
Cherry Pie	135	49.4	4.6	21.3	Gingerbread	100	57.8	4.2	7.6
Crunchy Cookies	75	53.8	3.7	15.1	Refrigerator Cookies	60	34.9	4.2	20.9
		382.6	68.6	110.4	Molasses Cookies	50	32.4	2.8	7.7
							403.7	68.2	109.2

Table 2 (Cont.)

Item	Diet VII E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Orange	190	21.3	1.8	0.4
Strawberries	100	27.9	0.6	0.2
Apple	230	25.3	0.5	0.2
Jelly	10	6.5	--	--
Lettuce	40	1.2	0.4	--
Radish	10	0.4	0.1	--
Potato Chips	20	9.8	1.4	7.4
Carrots (canned)	75	4.8	0.5	0.4
Baked Potato	100	19.1	2.1	0.1
Milk	240	11.8	8.4	9.4
Butter	40	--	0.4	32.4
Cube Steak	50	--	12.8	9.3
Ham	40	--	7.4	4.0
Sweet Roll	130	70.1	9.5	15.3
Bread	150	78.9	13.4	5.3
White Cake	100	57.4	6.3	12.2
Crunchy Cookies	75	53.8	3.7	15.1
		388.3	69.2	111.7

[1] The composition of the diets represent the calculated values obtained from tables of food composition.

[2] The composition of the saltine crackers obtained from the National Biscuit Co. Research Center, 2111 Route 208, Fair Lawn, New Jersey.

[3] E.P. represents the edible portion of the food items.

Table 3

The composition of the foods comprising the experimental diets.[1]

Diet I					Diet II				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Grapefruit Juice	120	12.4	0.6	0.1	Orange Sections	100	11.1	0.9	0.2
Pears (canned)	115	21.1	0.2	0.1	Jelly	20	13.0	--	--
Jelly	25	16.2	--	--	Vegetable Chop Suey	350	18.8	4.3	0.5
Vegetarian Stew	75	8.0	1.4	3.3	Tomato Slices	50	2.0	0.5	0.2
Tomato/Rice Casserole	245	22.3	3.2	3.8	Lettuce	50	1.4	0.6	0.1
Lettuce Wedge	50	1.4	0.6	0.1	Green Pepper	20	1.2	0.2	--
Butter (protein free)	45	--	--	36.0	Potato Salad	75	12.8	1.3	5.8
Pettijohn Cereal	180	24.2	3.0	0.7	Butter (protein free)	40	--	--	32.0
Dumplings (raw wt.)	120	45.2	7.8	3.6	Farina	250	33.8	4.3	0.3
Bread	475	249.7	42.4	16.8	Chow Mein Noodles	38	22.0	5.0	9.0
Peach Crumb Pie	135	68.0	3.4	12.6	Bread	450	236.6	40.2	15.9
Molasses Cookies	50	32.4	2.8	7.7	Pineapple Upside Down Cake	75	41.4	2.0	9.8
		500.9	65.4	84.8	Gingerbread	100	57.8	4.2	7.6
					Crunchy Cookies	25	17.9	1.2	5.0
							469.8	64.7	86.4

Table 3 (Cont.)

Diet III					Diet IV				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Strawberries	100	27.9	0.6	0.1	Orange Juice	100	10.9	0.8	--
Fruit Plate	227	44.5	1.5	0.3	Melon Balls	75	11.8	0.4	0.1
Jelly	30	19.5	--	--	Jelly	30	19.5	--	--
Shredded Cabbage/ Pineapple Salad	50	2.7	0.7	0.1	Lettuce Wedge	25	0.7	0.3	--
Spaghetti Sauce	200	9.6	2.0	5.0	Vegetable Soup	250	9.8	1.5	3.0
Butter (protein free)	40	--	--	32.0	Creole Green Beans	170	16.9	3.1	3.6
Pettijohn	180	24.2	3.0	0.7	Butter (protein free)	50	--	--	40.0
Bread	450	236.6	40.2	15.9	Cream of Wheat	235	65.9	5.1	0.5
Spaghetti	146	44.1	7.4	0.9	Bread	475	249.7	42.4	16.8
Apple Pie	130	39.7	3.0	15.0	Cherry Kuchen	100	45.6	3.3	12.6
Refrigerator Cookies	50	29.0	3.4	17.3	Molasses Cookies	50	32.4	2.8	7.7
		477.8	61.8	87.3	Ginger Cookies	75	50.1	4.7	11.5
							513.3	64.4	95.8

Table 3 (Cont.)

Diet V					Diet VI				
Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.	Item	E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Orange Juice	100	10.9	0.8	--	Grapefruit Sections	100	18.5	0.5	0.2
Apricots (canned)	120	21.4	0.6	0.1	Fruit Ice	100	36.0	0.5	0.1
Fruit Salad	50	8.2	0.3	0.1	Jelly	30	19.5	--	--
Jelly	20	13.0	--	--	V-8 Juice	100	4.3	1.0	0.2
Tomato Slices	50	2.0	0.5	0.2	Lettuce	25	0.7	0.3	--
Cucumber Slices	25	0.7	0.2	--	Pizza Sauce	175	8.2	2.0	1.7
Lettuce	50	1.4	0.6	0.1	Butter (protein-free)	40	--	--	32.0
Boiled Dinner	150	10.1	1.4	0.3	Cream of Wheat	177	50.5	3.9	0.4
Butter (protein free)	40	--	--	32.0	Bread	325	170.9	29.0	11.5
Wheatena	270	37.2	5.0	1.0	Macaroni Salad	240	48.7	7.9	16.1
Bread	500	262.9	44.7	17.6	Pizza Dough (raw wt.)	150	65.5	11.3	3.5
Saltine Crackers[2]	25	18.1	2.2	3.1	Cherry Pie	130	46.4	4.3	20.0
Applesauce Cake	100	59.9	3.6	16.2	Refrigerator Cookies	25	14.5	1.7	8.7
Crunchy Cookies	50	35.9	2.5	10.0			483.7	62.4	94.4
		481.7	62.4	80.7					

Table 3 (Cont.)

Item	Diet VII E.P.[3] gm.	CHO gm.	Pro gm.	Fat gm.
Orange	100	11.2	0.9	0.3
Strawberries	50	13.9	0.3	0.1
Apple	150	16.5	0.3	0.2
Lettuce, 30 gm. Radish, 10 gm.	40	1.3	0.4	1.0
Cucumber Slices	25	0.7	0.2	--
Lettuce	25	0.7	0.3	--
Baked Potato	100	19.1	2.0	0.1
Fr. Cut Green Beans	100	6.1	1.7	0.1
Butter (protein free)	40	--	--	32.0
Sweet Rolls	200	107.5	14.5	23.3
Bread	400	210.3	35.7	14.1
Shortcake	50	22.8	3.4	8.3
Applesauce Cake	75	44.9	2.7	12.1
Ginger Cookies	25	16.7	1.6	3.8
		471.7	64.0	95.4

1 The composition of the diets represent the calculated values obtained from tables of food composition.

2 The composition of the saltine crackers obtained from the National Biscuit Co. Research Center, 2111 Route 208, Fair Lawn, New Jersey.

3 E.P. represents the edible portion of the food items.

Table 4

Menus for the control period.

The Roman numerals refer to the different diets which are listed in Table 2. Complements are the items which the subjects could use with one or all meals for that day.

Meal[1]	I	gm.	II	gm.
B	Grapefruit **Juice**	150	Orange Juice	150
	Puffed Rice	14	Oatmeal	236
	Toast	50	Toast	50
	Milk	240	Milk	240
L	Spanish Rice	300	Onion Soup (canned)	200
	French Bread	50	Saltine Crackers	25
	Relish Plate - Celery	25	Lettuce	50
	Carrot	25	Tomato	50
	Lime Sherbet	75	Bread	100
	Ginger Cookies	50	Potato Chips	20
			Cherry Kuchen	100
D	Roast Beef	50	Pork Chop	60
	Oven **Browned** Potatoes	100	Bread Dressing	150
	Green Beans (canned)	75	Carrots (canned)	75
	Lettuce Wedge	50	Shredded Cabbage/ Pineapple Salad	50
	Hot Rolls	100	Hot Rolls	50
	Peach Crumb Pie	135	Applesauce Cake	75
C	Butter	45	Butter	50
	Jelly	20	Jelly	20
S	Refrigerator Cookies	60	Crunchy Cookies	50

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

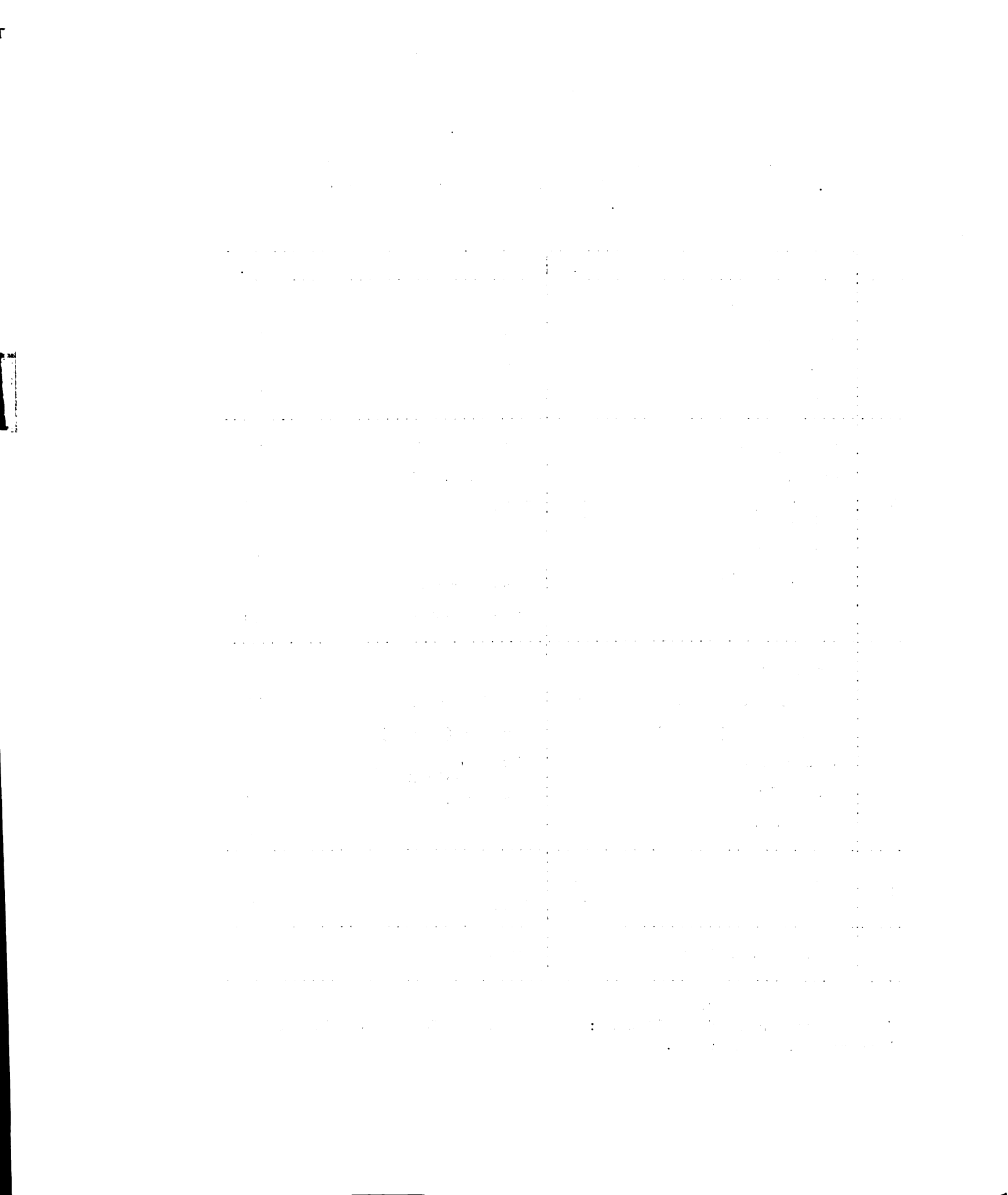

Table 4 (Cont.)

Meal[1]	III	gm.	IV	gm.
B	Grapefruit Juice	185	Orange Juice	150
	Shredded Wheat Biscuit	22	Sweet Rolls	130
	Toast	50	Rice Krispies	28
	Milk	240	Milk	240
L	Beef Patty	40	Potatoes au Gratin	130
	Bun	38	Bread Crumbs	20
	Pickles -Dill	30	Asparagus Spears	50
	Catsup	15	Mixed Salad	
	Mustard	10	Lettuce	20
	French Fries	50	Green Pepper	10
	Apricots (canned)	120	Tomato	20
	Cupcake	50	Bread	50
	White Icing	30	Melon Balls	100
			Molasses Cookies	40
D	Roast Chicken	40	Canadian Bacon	50
	Steamed Rice	100	Harvard Beets	100
	Stewed Tomatoes (canned)	100	Pineapple/Carrot Salad	50
	Lettuce	30	Hot Rolls	100
	Green Pepper	20	Chocolate Cake	100
	Hot Rolls	50		
	Lemon Pie/Meringue	160		
C	Butter	50	Butter	40
	Jelly	20	Jelly	20
S	Refrigerator Cookies	50	Ginger Cookies	50

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

Table 4 (Cont.)

Meal[1]	V	gm.	VI	gm.
B	Sliced Banana	100	Grapefruit Sections	100
	Corn Flakes	25	Puffed Wheat	12
	Toast	50	Toast	50
	Milk	240	Milk	240
L	Vegetable Soup (canned)	200	Creole Spaghetti Sauce	100
	Saltine Crackers	25	Spaghetti	100
	Peanut Butter	20	French Bread	100
	Jelly	30	Pears (canned)	115
	Bread	75	Refrigerator Cookies	60
	Celery Sticks	25		
	Apple	230		
D	Baked White fish(raw wt.)	60	Veal Cutlet	40
	Butter	5	Boiled Potatoes	75
	Mashed Potatoes	100	Fr. Cut Green Beans	100
	Peas	50	Fruit Salad	50
	Lettuce Wedge	50	Lettuce	20
	Hot Rolls	75	Hot Rolls	75
	Cherry Pie	135	Gingerbread	100
			Lemon Sauce	50
C	Butter	40	Butter	50
	Jelly	20	Jelly	30
S	Crunchy Cookies	75	Molasses Cookies	50

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, D; Snack, S.

Table 4 (Cont.)

Meal [1]	VII	gm.
B	Orange	190
	Sweet Roll	130
L	Cube Steak	50
	Baked Potato	100
	Whole Carrots (canned)	75
	Lettuce	40
	Radish	10
	Hot Rolls	100
	Milk	240
	White Cake	100
	Strawberries	100
D	Ham	40
	Bread	50
	Potato Chips	20
	Crunchy Cookies	75
	Apple	230
C	Butter	40
	Jelly	10

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C.

Table 5

Menus for the experimental period.

The Roman numerals refer to the different diets which are listed in Table 3. Complements are the items which the subjects could use with one or all meals for that day.

Meal [1]	I		II	
B	Grapefruit Juice	120	Orange Sections	100
	Pettijohn	180	Farina	250
	Toast	100	Toast	100
L	Dumpling (raw wt.)	120	Tomato Slices	50
	Vegetable Stew	75	Lettuce	30
	Bread	100	Potato Salad	75
	Pears (canned)	115	Bread	150
	Molasses Cookies	50	Gingerbread	100
D	Tomato/Rice Casserole	245	Vegetable Chop Suey	350
	Bread Crumbs/Butter	30	Chow Mein Noodles	38
	Lettuce	50	Lettuce/Green Pepper	40
	Hot Rolls	200	Hot Rolls	200
	Peach Crumb Pie	135	Pineapple Upside Down Cake	75
C	Butter (protein free)	40	Butter (protein free)	40
	Jelly	10	Jelly	20
S	Bread	50	Crunchy Cookies	25
	Jelly	15		

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

Table 5 (Cont.)

Meal[1]	III	gm.	IV	gm.
B	Strawberries	100	Orange Juice	100
	Pettijohn	180	Cream of Wheat	235
	Toast	100	Toast	125
L	Fruit Plate	227	Vegetable Soup	250
	Bread	150	Bread	150
	Refrigerator Cookies	50	Melon Balls	75
			Molasses Cookies	50
D	Spaghetti	146	Creole Green Beans	170
	Spaghetti Sauce	200	Lettuce Wedge	25
	Sh. Cabbage/Pineapple	50	Hot Rolls	200
	French Bread	150	Cherry Kuchen	100
	Apple Pie	130		
C	Butter (protein free)	40	Butter (protein free)	50
	Jelly	15	Jelly	30
S	Bread	50	Ginger Cookies	75
	Jelly	15		

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

Table 5 (Cont.)

Meal[1]	V	gm.	VI	gm.
B	Orange Juice	100	Grapefruit Sections	100
	Wheatena	270	Cream of Wheat	177
	Toast	150	Toast	100
L	Tomato Slices	50	V-8 Juice	100
	Cucumber Slices	25	Macaroni Salad	240
	Lettuce	30	Bread	100
	Bread	150	Cherry Pie	130
	Apricots (canned)	120		
	Crunchy Cookies	50		
D	Boiled Dinner	150	Pizza Dough (raw wt.)	150
	Fruit Salad	50	Pizza Sauce	175
	Lettuce	20	Lettuce	25
	Hot Rolls	200	French Bread	100
	Applesauce Cake	100	Fruit Ice	100
			Refrigerator Cookies	25
C	Butter (protein free)	40	Butter (protein free)	40
	Jelly	20	Jelly	15
S	Saltine Crackers	25	Bread	25
			Jelly	15

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

Table 5 (Cont.)

Meal[1]	VII	gm.
B	Orange	100
	Sweet Rolls	200
	Bread	50
L	Baked Potato	100
	Fr. Cut Green Beans	100
	Chef's Salad -Lettuce	30
	Radish	10
	Hot Rolls	200
	Shortcake	50
	Strawberries	50
D	Cucumber Slices	25
	Lettuce	25
	Bread	150
	Applesauce Cake	75
	Apple	150
C	Butter (protein free)	40
S	Ginger Cookies	25

[1] The letters denote the following: Breakfast, B; Lunch, L; Dinner, D; Complements, C; Snack, S.

RESULTS AND DISCUSSION

Coons (1930) suggested that if the experimental diet is a drastic change from the control diet a period should be allowed for the subjects to establish equilibrium on the new regimen. No transitional period was required by the "bread study" since the experimental diet contained approximately the same amount of amino acids as the control. Because of the low level of nitrogen in the experimental diets, the control period was employed so that the subjects could attain equilibrium. The objectives of the study were to determine whether or not the subjects could maintain nitrogen equilibrium on a diet in which 90 per cent of the protein was from wheat flour. Reifenstein et al., (1945) warned against the interpretation of a balance study when the subjects are placed on a diet which is a change from the normal intake.

It has been pointed out that the length of a study is determined by the objectives of the project. Donelson et al., (1931) stressed the fallacies which a researcher can depict by interpreting a study that is too short to reasonably fulfill its purpose. The interpretation of long balance studies can also reveal results which are not possible (Walker,1962). The twenty-one day control period allowed the boys to attain equilibrium on the moderately low protein diet.

In the literature there are no statements concerning the relationship between the personnel of a study and the subjects. It is feasible to assume that a rapport, as discussed by Leichsenring et al., (1958), should be established so that the subject will admit any errors committed. The errors in the "bread study" that were reported by the subjects were eating food other than that allowed, collecting only some of the excreta and not recording the ad libitum items. From the

observations of this study it can be hypothesized that the personnel should develop rapport yet remain detached from the subjects. This was apparent when breakfast was served. The serving time of breakfast was from 7:15 to 8:30. There were several occasions when the morning cook would tell one subject that it would be permissible if he came in later than the set time. When this was discovered, stern comment was required by the research staff in order to stress the importance of the meal serving time. After several encounters the problem was improved and the subject reported to breakfast within the set serving hours.

The trial period that was carried out prior to this study did clarify the working procedures of the cooks. However, because the participants were not the same people who were employed in the study, the remaining purposes of such a period were not fulfilled. These were a development of rapport between the staff and the subjects and an adjustment by the subjects to the low protein diet and to the routines of the study. Thus, the trial period seemed to have little merit. The control period met the objectives of the trial period. Because of this factor it seems advisable to use part of the control period as the trial period. Leichsenring et al., (1958) reported that during the trial period analyses need not be performed. This is not true, for it is important that the personnel involved in the analytical laboratory develop reliable analytical techniques and also adapt to the demands of the study. Also, if the constituents of the diets are determined, the reliability of the computed diets can be evaluated.

It has been pointed out that there is less supervision of the subjects in a non-clinical study. Bauer and Aub (1927) stressed the importance of control of the subjects throughout the study, whereas,

McKay et al., (1942) stated that the environment plays an important role in the results of the study. Therefore, the latter authors report that the environment should be, as much as possible, the normal living situation to which the particular subjects are accustomed. Throughout this study the only supervision the twelve subjects received was during the meal serving periods and during the various tests to which they were subjected. It was believed that the subjects were trustworthy and honest throughout the study. To create a home-like atmosphere during the meal service the subjects picked up their own hot food from the cooks in the kitchen and their cold food dishes from the tables in the dining room.

According to Johnston and McMillan (1951) the only fresh fruit to be employed in metabolic studies is citrus fruit. The other fruits and vegetables should be frozen or canned. The research with which the authors were concerned was the investigation of the absorption of iron from spinach. The "bread study" was mainly concerned with nitrogen intake from wheat products. Since the fruits and vegetables chosen for the study contributed only a negligible amount of nitrogen, the use of only frozen and canned fruits or vegetables was not followed.

The food items (Tables 2 and 3) consisted of many mixed dishes and desserts. They were used due to the length of the study, the subject's emotional aspects, and the objectives of the study. Because no meat or dairy products were allowed in the experimental diet, an entree was impossible to prepare without the use of mixed dishes. If mixed dishes had not been employed, the subjects would have received several separate dishes of individual vegetables. Spaghetti and macaroni could have been presented with the vegetables. This type of diet is bland and unattractive. For fifty days the subject had to be presented food that was appealing and

appetizing. Most of the mixed dishes contributed only a small amount of nitrogen to the total diet. The emotional disturbances which might be produced by forcing the subject to eat undesired food could yield results which would not be a direct effect of the diet but rather due to the subject's emotional conflicts. Schottstaedt and co-workers (1958) showed that there were metabolic changes in the excretion of nitrogen when the subjects were under stressful conditions.

Sampson et al., (1952) implied that desserts such as cake and pie are not to be incorporated into metabolic balance study menus. The researcher must avoid such a statement without the consideration of the objectives of the study. During the experimental phase 90 to 95 per cent of the protein was derived from wheat products. One loaf of bread per day was consumed by each subject. They also received cake, pie, cookies, cereals, and some entrees which contained wheat products. These foods were required to attain the level of the type of protein required by the study. Such foods could not have been used if the prime objective of this study had been the determination of fat and calories. These were determined but only for the purpose of knowing the amount consumed by the subjects.

In calculating the nitrogen intake of each subject from coffee and tea several problems were encountered. Because the coffee was weighed only at the beginning and end of the study, it was impossible to determine the amount consumed by each subject in a balance period. The total weight as obtained from weighing the returned coffee at the end of the study did not in 75 per cent of the cases equal the amount recorded by the subjects. In these instances the amount recorded by the subjects was too low. Since tea bags were employed, it was impossible to determine the exact strength of tea consumed. As the strength of the tea

increases, the amount of nitrogen increases.[1] In future nitrogen balance studies the instant coffee and tea should be weighed at the beginning and end of each balance period in order to determine the exact amount consumed by the subject. With the coffee and tea, sugar was dispensed to the subjects in individual packets. These were easy to handle and the amount of sugar as listed on each packet allowed for accurate recording.

Maintaining the weights of the subjects during the experimental regimen required excessive amounts of whey-free (protein-free) butter, jelly, sugar and honey per day. The amounts of these complements were as much as 150, 100, 50, and 35 grams of butter, jelly, honey and sugar, respectively, per day. The whey-free butter contributed many calories to the diet of these individuals without increasing their nitrogen intake. No weight gain was observed in the individuals losing weight even when the intake was 4300 calories per day. In some subjects there was a slight continual loss of weight even at this caloric level. The weather at the beginning of the study was cold, however, after the experimental regimen began the weather moderated. The subjects began to increase their physical activity with this change of weather. Bricker et al., (1949) suggested an experimental design in which there is a control group throughout the experiment. Because the facilities for such a study were inadequate on this campus, this design could not be followed. However, had it been employed more valid assumptions could be made. If the weight loss had been observed in both the experimental and control groups during the same weather conditions, the weight loss could be attributed with more certainty to the change in weather which in turn increased the physical activity. There were three subjects who showed

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[1] The analyses of the tea bags when placed in 170 ml. boiling water for one-half, one, and one and one-half minutes revealed 0.014, 0.024, and 0.027 gm. of N, respectively.

weight gains. One subject received only dietetic jelly and small amounts of sugar after the first week of the experimental regimen in order to keep his weight gain to a minimal.

When requesting food dislikes of an individual some times they cannot all be recalled by the subject. Such a case occurred in this experiment. One of the subjects disapproved of Harvard Beets. However, he did not note this on the questionnaire. Harvard Beets were included in control diet IV (Table 4). The subject's dislike for these was conveyed to the other participants and aroused some emotional disturbance within the group. This observation agrees with that of Marble (1939), Presson (1955), and Sampson and co-workers (1952). The importance of the listing should be stressed to the potential subjects of a balance study. Many people think they can tolerate any food. However, when the subject is placed on a control regimen and is required to eat each dish at least once a week, the tolerance the subject stated that he had for the food, lessens.

The Hanson spring scale according to Widdowson and McCance (1942) and Coons (1930) is accurate to one gram. The accuracy of this scale and the Toledo scale was determined by weighing a specified gram weight eight successive times. Each time that the weight was removed the indicator on the Hanson scale was returned to zero (Refer to Table 6).

It is true that the Hanson scale does weigh accurately to one gram. However, the per cent error for the various weights must be considered. The results show that there is an increase in the per cent of error for the smaller weights for both scales. The Hanson scale shows an underestimation for all weights except for the 100 gram weight. This weight on the Hanson scale reveals less deviation than the same weight on the

Table 6

The validity of the scales employed in the metabolic kitchen.

Hanson spring scale				
	gm. weight			
Weighing	10	20	50	100
1	9.0	19.0	49.5	100.0
2	10.0	18.5	50.0	99.5
3	9.0	19.0	50.0	100.0
4	10.0	19.5	50.0	100.5
5	10.0	19.0	50.0	100.0
6	9.5	19.0	50.0	100.0
7	9.5	19.0	49.5	100.0
8	9.5	19.0	49.0	100.0
Delta Mean Deviation	0.44	0.88	0.25	0.13
Per Cent Error	4.4	4.4	0.50	0.13

Toledo scale				
	gm. weight			
Weighing	100	200	1200	3200
1	101.0	201.0	1200.5	3201.5
2	100.0	200.5	1200.5	3201.0
3	100.5	200.5	1200.5	3201.0
4	100.5	201.0	1200.0	3201.0
5	100.0	200.5	1200.5	3201.0
6	100.5	200.5	1200.0	3201.0
7	100.0	200.5	1200.5	3201.0
8	100.0	200.0	1200.5	3201.0
Delta Mean Deviation	0.31	0.56	0.38	1.06
Per Cent Error	0.31	0.28	0.03	0.03

Toledo scale. It is possible to say that for weighing small items (less than 50 grams) where less than 5.0 per cent error is desired, the Hanson scale should not be employed.

The food items served which weighed less than 50 grams and which were weighed on the Hanson scale in both regimens were butter, jelly, sugar, salads, saltine crackers, dry cereals, bread, meat and cookies. The meat and dry cereals were served during the control diet. The remaining foods listed are low in nitrogen or nitrogen-free with the exception of bread, cookies and saltine crackers. The majority of these items were greater than 50 grams and none of these were below 25 grams. The ingredients of the recipes which weighed less than 100 grams were weighed on the Hanson scale, whereas, for those greater than 100 grams the Toledo scale was employed. Only in one of the recipes did the items that contributed to the source of wheat protein weigh less than 40 grams and only in two recipes were the weights of the wheat products less than 56 grams. Since the nitrogen contributing items were greater than 50 grams throughout most of the experiment, the error induced by the employment of these scales was negligible. The scales were checked for accuracy periodically throughout the study with weights.

Upon using food tables to calculate the nutritive value of the diets an error caused by a misrepresentation of the protein value of flour occurred. The amount of protein in all purpose, enriched flour as listed in the U.S.D.A. Handbook No. 8 (Watt and Merrill, 1963) is 10.5 per cent. This figure was employed in calculating the diets. Analysis of the flour used in the study revealed a value of 12.8 per cent protein. This figure was based on the nitrogen analysis by the macro-Kjeldhal

method. The protein was calculated by multiplying the nitrogen by the conversion factor, 5.70 (Jones, 1931). The "high protein" flour used in the study was Gold Medal Kitchen Tested, enriched, all purpose, brominated "Better for Bread." This was introduced by General Mills in 1953 (Celender, Personal Communication, 1964). It is packed only in 25, 50, and 100 pound bags and its distribution is primarily in areas where there is home bread baking. Its protein content ranges from 12.0 to 13.0 per cent. When 12.8 per cent was employed to calculate the protein in the flour, a closer correlation was observed between the calculated and analyzed values for the diets. The average calculated values using the higher protein figure for the flour revealed 68.4 and 63.7 grams of protein for the control and experimental diets, respectively. The analyzed average values for the seven diets were 64.4 and 63.3 grams of protein, respectively, for the control and the experimental regimens.

The flour was calculated to contain the following by weight:

Water	12.0 per cent
Protein	12.8* per cent
Carbohydrate	73.8 per cent
Fat	1.0 per cent
Ash	0.4 per cent

* represents analysis from this laboratory

The error introduced by employing the protein value listed in the food tables for flour demonstrates the inadequacy of such tables. They only can be assumed to present the approximate nutritive value of the items.

The results of the two methods employed in calculating the energy content of the diets can be observed in Table 7. If the heat of combustion values are employed, there is a closer agreement with the analyzed values. It can be observed that if the 4, 9, 4 values, which represent the

Table 7

Comparison of two methods to determine the energy value of the diets.

Diet	General Factors [1]	Specific Factors [2]	Anal. Calories [3]	Diet	General Factors [1]	Specific Factors [2]	Anal. Calories [3]
Control I	2848	3052	3085	Exptl. I	3029	3258	3288
Control II	2961	3184	3240	Exptl. II	2916	3142	3130
Control III	2887	3095	2856	Exptl. III	2943	3154	3047
Control IV	2934	3150	3031	Exptl. IV	3174	3408	3294
Control V	2798	2999	2977	Exptl. V	2902	3121	3151
Control VI	2870	3082	2772	Exptl. VI	3034	3253	3107
Control VII	2835	3043	2920	Exptl. VII	3002	3228	3176
Mean	2876	3086	2983		3000	3237	3176

[1] The general factors employed to calculate the energy value of the diets were 4, 9, 4, calories per gram of carbohydrate, fat, and protein, respectively.

[2] The specific factors employed were those as recommended by Atwater (1899). These factors represent the heat of combustion values for the individual food groups (Refer to pp. 43 - 44).

[3] The values for the control diets represent the averages of the duplicate analyses of the same diet collected at zero and one and one-half hour intervals after the food was prepared. The values for the experimental diets represent the average of the duplicate analyses of the same diet collected at zero hour for each of four successive weeks.

available physiological food energy per gram of carbohydrate, fat, and protein, respectively are employed errors can result. Thus, to make a more valid comparison, the correct heat of combustion factors must be used.

Jones (1931) criticized the protein values of foods listed in the food tables. If the food tables do not take into consideration the two assumptions that are assumed in nitrogen determinations, the protein value represented could be false. These assumptions are: (1) that all protein contains 16 per cent nitrogen which accounts for the conversion factor of 6.25, and (2) that all the nitrogen in the food is protein.

The U. S. D. A. food composition tables do consider these assumptions. The protein values reported therein (Mayer, 1952) represent those calculated using the specific factors for converting the analyzed nitrogen to protein. The effect of using the wrong conversion factor to determine the amount of nitrogen in the control diet from the calculated protein figure can be seen in Table 8. Since the control diets contained only a minute amount of animal protein, the value of 6.25 which is employed to determine the protein intake in most American diets, could produce false values. However, when the specific factors for the food groups are employed, a closer correlation between the calculated and analyzed nitrogen is secured. In the experimental diets a different picture is presented. The factors 5.70 and 6.25 were used to convert calculated protein from the wheat products and fruit and vegetables, respectively. Since such a small amount of protein came from foods other than those containing wheat, it did not matter whether 5.70 was used exclusively or the specific factors were employed. Both factors

Table 8

Comparison of dietary nitrogen calculated using various factors for converting the protein values listed in tables of food composition with the analyzed nitrogen values.

Diet	Calcd. N (gm.)		Analyzed
	Reg. C. F.[1]	Specific C. F.[2]	N (gm.)[3]
Control I	10.6	11.1	11.4
Control II	11.3	11.9	12.9
Control III	10.7	11.0	10.8
Control IV	10.8	11.4	11.0
Control V	11.0	11.6	10.7
Control VI	10.9	11.4	11.2
Control VII	10.9	11.5	11.4
Mean	10.9	11.4	11.3
Exptl. I	11.5	11.4	11.8
Exptl. II	11.4	11.3	11.1
Exptl. III	10.8	10.7	10.7
Exptl. IV	11.3	11.2	11.2
Exptl. V	10.9	10.9	11.2
Exptl. VI	10.9	10.8	10.9
Exptl. VII	11.2	11.2	11.1
Mean	11.1	11.1	11.1

[1] The regular conversion factor used for the control diets was 6.25; that for the experimental diets was 5.70 which was proposed by Jones (1931) for wheat and cereal products.

[2] The specific conversion factors proposed by Jones (1931) were applied to each food group. (Refer to p. 44).

[3] The control diet's nitrogen determination represents the average of the analyses on the same diet collected at zero hour and one and one-half hour after the food was prepared. The experimental diet's nitrogen determination represents the average of the analyses on the same diet collected at zero hour for each of four successive weeks.

resulted in a close agreement between the calculated values and the determined nitrogen content.

The reliability of food tables was demonstrated when the calculated and analyzed values of the diets were compared. Table 9 shows that the calculated nitrogen fell within 5 per cent of the analyzed values in five out of the seven control diets. Diet V reveals that the calculated nitrogen is 8.4 per cent greater than the analyzed, whereas, diet II discloses that the analyzed value is 7.8 per cent greater than the calculated. The calculated fat value of diet I is within 3.5 per cent, diet II is within 5 per cent, and all the other diets (III-VII) reveal that the calculated fat is greater than 10 per cent of the analyzed values. The determined food energy is within 5 per cent for five of the seven calculated values. Diets III and VI show calculated calories to be greater than 10 per cent of the analyzed values.

The nitrogen data is in agreement with Lutwak et al., (1964) and Widdowson and McCance (1943). In their studies they reported that the calculated nitrogen was within 10 per cent of the analyzed. Kaucher and co-workers (1945) found in their studies a close comparison between the calculated and analyzed values for food energy. However, the values for fat were 14 per cent higher than the analyzed. The calculated fat values in the "bread study" are 14 per cent and 13 per cent higher than the analyzed for the control and experimental diets, respectively.

In observing diet III of the control menu in Table 4, there are two food items exclusive of butter which contained large amounts of fat. These are lemon pie/meringue and refrigerator cookies. The fat probably was not distributed homogeneously in these food items which would lead to error. Hawks et al., (1937) and Thomas et al., (1950) stated that it

Table 9

Comparison of calculated and analyzed values for nitrogen, fat, and calories in the control diets collected at two times during the meal service.

DIET	NITROGEN (Gm.)				FAT (Gm.)				ENERGY (CALORIES)			
	Calc.[1]	Anal.[2]	Δ	Δ as % Anal.	Calc.	Anal.[2]	Δ	Δ as % Anal.	Calc.[3]	Anal.[2]	Δ	Δ as % Anal.
I	11.1	11.4	+ .3	2.6	111.7	107.9	- 3.8	3.5	3052	3085	+ 33	1.1
II	11.9	12.9	+1.0	7.8	131.5	125.2	- 6.3	5.0	3184	3240	+ 56	1.7
III	11.0	10.8	-0.2	1.8	121.1	105.0	-16.1	15.3	3095	2856	-345	12.1
IV	11.4	11.0	-0.4	3.5	113.9	95.1	-18.8	19.8	3150	3031	-119	3.9
V	11.6	10.7	-0.9	8.4	110.4	93.4	-17.0	18.2	2999	2977	- 22	0.74
VI	11.4	11.2	-0.2	1.8	109.2	91.2	-18.0	19.7	3082	2772	-310	11.2
VII	11.5	11.4	-0.1	0.88	111.7	98.3	-13.4	13.6	3043	2920	-123	4.2
Mean	11.4	11.3	0.4		115.6	102.3	13.3		3086	2983	144	
Delta Mean	0.21	0.47	0.27		6.1	8.9	4.8		48	115	105	
S.D.	0.35	0.6	0.3		8.0	11.8	5.9		64	153	138	
	t =0.294 P =<.8				t = 2.29 P = <.05				t = 1.41 P = <.2			

[1] Nitrogen represents the protein content calculated from the U.S.D.A. Hdbk. No. 8 (Watt and Merrill, 1950 and 1963) divided by the conversion factors for each food group as prescribed by Jones (1931). (Refer to p. 44).

[2] The values representing nitrogen, fat, and energy analyses are based on the average of the values obtained from duplicate analyses of diets collected at zero and one and one-half hour after the food was prepared.

[3] Calories computed by employing Atwater's (1899) heat of combustion factors (Refer to pp. 43-44).

is impossible to collect a sample for analysis which is homogeneous. The latter author further emphasized that no two servings of a food which is prepared in one large receptacle can contain the same components. Diet IV also contains two items which are high in fat content; they are sweet rolls and chocolate cake. The milk and the meats which were all lean and trimmed of fat prior to cooking can be considered homogeneous in their fat content when compared to the mixed dishes. The desserts in diet V could also be subject to variation in the amount of fat dispersed in each serving. Refrigerator cookies appear again in diet VI. They were served in diet I but the difference produced in the analyzed versus calculated fat was not affected. Diet VII food items which could contribute to the differences in calculated versus analyzed fat are sweet rolls, white cake, and crunchy cookies. The desserts and the bread which could play a role in the fat differences were prepared with the aid of an electric mixer in order to make the foods as homogeneous as possible. The inability to disperse the fat evenly in the foods could also account for the differences between the calculated and analyzed values for calories. However, these differences are less than for fat, but the trends are the same except for diets I and II where the calculated values are higher than the analyzed.

Similar results are found for the experimental diets (Table 10). All but four of the diets calculated are within 5 per cent of the analyzed values for nitrogen. The diets not within this range are IA, IIB, VID, and VIIB.[1] According to the literature

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[1] The capital letters denote the four successive weeks of collected diet samples: A, week 1; B, week 2; C, week 3; and D, week 4.

Table 10

Comparison of calculated and analyzed values for nitrogen, fat, and calories in the seven experimental diets collected during four successive weeks.

Diet	NITROGEN (Gm.)				FAT (Gm.)				ENERGY (CALORIES)			
	Calc.[1]	Anal.[3]	Δ	Δ as % Anal.	Calc.	Anal.[3]	Δ	Δ as % Anal.	Calc.[2]	Anal.[3]	Δ	Δ as % Anal.
IA	11.4	12.1	+0.7	5.8	84.8	68.1	-16.7	24.5	3259	3282	+33	0.7
IB	11.4	11.8	+0.4	3.4	84.8	78.5	- 6.3	8.0	3259	3322	+63	1.9
IC	11.4	11.8	+0.4	3.4	84.8	76.5	- 8.3	10.8	3259	3256[4]	- 3	0.09
ID	11.4	11.5	+0.1	0.87	84.8	73.9	-10.9	14.8	3259	3292	-33	1.0
Mean		11.8	0.4			74.3	11.6			3288	31	
Delta Mean		0.15	.15			3.4	3.6			19	17.5	
S.D.		0.24	.24			4.5	4.7			27	25	
	t = 2.67				t = 4.04				t = 1.76			
	P = <.05				P = <.01				P = <.20			
IIA	11.3	11.8	+0.5	4.2	86.4	70.5	-15.9	22.6	3242	3157	-85	2.7
IIB	11.3	10.6	-0.7	6.6	86.4	79.4	- 7.0	8.8	3242	3071	-171	5.6
IIC	11.3	11.0	-0.3	2.7	86.4	75.2	-11.2	14.9	3239[5]	3130	-109	3.5
IID	11.3	11.0	-0.3	2.7	86.4	84.6	- 1.8	2.1	3242	3161	- 81	2.6
Mean		11.1	0.45			77.4	9.0			3130	118	
Delta Mean		0.35	0.18			4.6	4.6			29	33	
S.D.		0.50	0.25			6.0	5.3			42	42	
	t = 0.667				t = 2.56				t = 3.42			
	P = <.6				P = <.05				P = <.02			

Table 10 (Cont.)

Diet	NITROGEN (Gm.)				FAT (Gm.)				ENERGY (CALORIES)			
	Calc.[1]	Anal.[3]	Δ	Δ as % Anal.	Calc.	Anal.[3]	Δ	Δ as % Anal.	Calc.[2]	Anal.[3]	Δ	Δ as % Anal.
IIIA	10.7	10.8	+0.1	0.92	87.3	84.5	- 2.8	3.3	3154	3066	- 88	2.9
IIIB	10.7	10.4	-0.3	2.9	87.3	71.6	-15.7	21.9	3154	2996	-158	5.3
IIIC	10.7	11.1	+0.4	3.6	87.3	66.0	-21.3	32.3	3154	3103	- 51	1.6
IIID	10.7	10.6	-0.1	0.92	87.3	70.1	-17.2	24.5	3154	3023	-131	4.3
Mean		10.7	0.21			73.1	14.3			3047	107	
Delta Mean		0.2	0.18			5.5	5.7			38	37.5	
S.D.		0.3	0.15			7.9	8.0			47	48	
	$t = 0.0$ $P = 1$				$t = 3.07$ $P = <.02$				$t = 3.47$ $P = <.02$			
IVA	11.2	11.1	-0.1	0.90	95.8	91.8	- 4.0	4.4	3408	3252	-156	4.8
IVB	11.2	11.1	-0.1	0.90	95.8	84.4	-11.4	13.5	3408	3252	-156	4.8
IVC	11.2	11.3	+0.1	0.88	95.8	85.2	-10.6	12.4	3412[6]	3314	- 98	3.0
IVD	11.2	11.2	0.0	0.0	95.8	88.4	- 7.4	8.4	3408	3357	- 51	1.5
Mean		11.2	0.08			87.5	8.4			3294	115	
Delta Mean		0.08	0.035			2.7	2.6			42	41	
S.D.		0.1	0.05			3.5	3.7			52	51	
	$t = 0.0$ $P = 1$				$t = 4.49$ $P = <.01$				$t = 4.33$ $P = <.01$			

Table 10 (Cont.)

Diet	NITROGEN (Gm.)				FAT (Gm.)				ENERGY (CALORIES)			
	Calc.[1]	Anal.[3]	Δ	Δ as % Anal.	Calc.	Anal.[3]	Δ	Δ as % Anal.	Calc.[2]	Anal.[3]	Δ	Δ as % Anal.
VA	10.9	11.0	+0.1	0.91	80.7	72.9[4]	- 7.8	10.7	3121	3002	-119	4.0
VB	10.9	11.4	+0.5	4.4	80.7	73.4	- 7.3	10.0	3121	3197	+ 76	2.4
VC	10.9	10.9	0.0	0.0	80.7	68.5	-12.2	17.8	3121	3207	+ 86	2.7
VD	10.9	11.4	+0.5	4.4	80.7	76.7	- 4.0	5.2	3121	3196	+ 75	2.4
Mean		11.2	0.3			72.9	7.8			3151	89	
Delta Mean		0.20	0.22			2.6	2.2			76	15	
S.D.		0.26	0.26			3.4	3.5			102	21	
	t = 2.4				t = 1.93				t =0.589			
	P = <.1				P = <.1				P = <.6			
VIA	10.8	10.7	-0.1	0.93	94.4	93.4	- 1.0	1.1	3253	3050	-203	6.7
VIB	10.8	10.6	-0.2	1.8	94.4	83.6	-10.8	12.9	3253	3115[4]	-138	4.4
VIC	10.8	10.7	-0.1	0.93	94.4	83.9	-10.5	12.5	3253	3106	-147	4.7
VID	10.8	11.4	+0.6	5.3	94.4	87.2	- 7.2	8.3	3219[7]	3157	- 52	1.6
Mean		10.9	0.3			87.0	7.4			3107	135	
Delta Mean		0.3	0.20			3.3	3.3			29	41	
S.D.		0.37	0.24			4.6	4.5			44	62	
	t =0.5				t = 3.02				t = 5.41			
	P =<.6				P = <.02				P = <.01			

Table 10 (Cont.)

Diet	Nitrogen Calc.[1]	Nitrogen Anal.[3]	Nitrogen Δ	Nitrogen Δ as % Anal.	Calories Calc.	Calories Anal.[3]	Calories Δ	Calories Δ as % Anal.	Calories Calc.[2]	Calories Anal.[3]	Calories Δ	Calories Δ as % Anal.
VIIA	11.2	11.3	+0.1	0.88	95.4	87.4	- 8.0	9.2	3228	3259	+ 31	0.95
VIIB	11.2	10.4	-0.8	7.7	95.4	70.0	-25.4	36.3	3228	2980	-338	11.7
VIIC	11.2	11.5	+0.3	2.6	95.4	89.8	- 5.6	6.2	3228	3426	+198	5.8
VIID	11.2	11.5	+0.3	2.6	95.4	83.6	-11.8	14.1	3228	3277	+ 49	1.5
Mean		11.1	0.4			82.7	12.7			3213	154	
Delta Mean		0.40	0.22			6.4	6.4			162	114	
S.D.		0.53	0.3			8.9	8.8			253	142	
	t =0.364				t = 2.57				t =0.112			
	P =<.8				P = <.05				P =>.9			
Avg.Mean	11.1	11.1			89.3	79.3			3237	3176		
	t = 0.0				t = 9.52				t = 2.81			
	P = 1				P = <.01				P = <.02			

[1] Nitrogen represents the protein content calculated from the U.S.D.A. Hdbk. No. 8 (Watt and Merrill, 1950 and 1963) divided by the conversion factors for each food group as prescribed by Jones (1931) (Refer to p. 44).

[2] Calories computed by employing Atwater's (1899) heat of combustion factors (Refer to pp. 43-44).

[3] All analyses represent the average of duplicate determinations on the samples.

[4] Calculated figures due to missing data. (Snedecor, 1946)

[5] 100 gm. orange juice substituted for 100 gm. orange sections in diet.

[6] 100 gm. orange sections substituted for 100 gm. orange juice in diet.

[7] 100 gm. grapefruit juice substituted for 100 gm. grapefruit sections.

(Leverton and Whiting, 1960) the accepted range for the calculated values for protein, fat, and calories to represent the determined amount is 10 per cent. Leverton and Whiting (1960) after reviewing three hundred cases in the literature, which compared calculated diets with the same diets analyzed, found 58 per cent and 54 per cent of the calculated values for calories and protein, respectively, to be within 10 per cent of the analyzed values; however, only 25 per cent of the calculated diets were within 10 per cent of the analyzed values for fat. The calculated nitrogen and energy values are within 10 per cent of the analyzed for 86 per cent of the experimental diets in the "bread study"; however, the calculated fat is within 10 per cent in 50 per cent of the analyzed diets. Leverton and Whiting (1960) found only 25 per cent of the calculated diets for fat to be within 10 per cent of the analyzed values . About half the calculated fat values were higher than the analyzed. In the "bread study" during both regimens the calculated values are higher than the analyzed in 100 per cent of the cases. For the experimental diets 71 per cent of the analyzed calories are below the calculated values. In reviewing the sources of fat in the diets, it is possible that the heterogeneity of the samples caused the deviation of the calculated fat and calories from the analyzed values.

Upon applying 3-way analysis of variance, no significance is found in the differences between the analyzed and calculated nitrogen values. The values from diet to diet are also not significant for the seven diets. The analyzed fat values show a significant difference from the calculated values at the 1 per cent level. There is also a 1 per cent significance among the seven analyzed diets when compared with the calculated values. The differences between the calculated-and analyzed food energy are

significant at the 5 per cent level. There is also a significant difference among the seven experimental diets at the 1 per cent level in the calculated versus the analyzed food energy. There is no significant differences for nitrogen, fat, and energy for the diets served one week to those in any other week. It can be assumed that the seven diets were the same in composition from one week to another.

These results suggest that the food table values for fat are not reliable. The calculated values for energy give slightly better agreement with the analyzed values than the fat values.

The "t" test for nitrogen in the control diets reveals a probability that the analyzed value will approximate the calculated value 80 per cent of the time (Table 9). For food energy there is only a 20 per cent probability that the analyzed value will approximate the calculated value. An even lesser probability is found with fats there being only a 5 per cent possibility that the determined value will represent the calculated figure as obtained from food tables. In the experimental diets similar results are found (Table 10). These are in agreement with the 3-way analysis of variance. The calculated nitrogen will represent the analyzed values nearly 100 per cent of the time. However, for fat, as well as food energy, there is only a 1 per cent and 5 per cent probability, respectively, that the determined value will represent the calculated value.

Upon observing the "t" test for the individual diets differences can be observed. The nitrogen is below 0.6 in two cases. These are diets I and V. This means that in some diets the calculated results are more reliable than in other diets. In diet I it can be postulated that the reason for a low probability is due to the dumpling and vegetarian stew

served at lunch. The dumpling dough was weighed upon mixing and placed in the stew. The subjects were then served one dumpling and 75 grams of the stew. Since the dumpling contributed approximately 10 per cent of the nitrogen, errors made by the kitchen personnel could have caused a difference in the amount of dumpling the sample received, thus causing significant differences in the amount of nitrogen in the total diet. In future studies entrees of this type should be eliminated. In diet V some variations could have been introduced by the boiled dinner served at dinner or the cereal served at breakfast. However, there is a high probability of the analyzed nitrogen approximating the calculated values in the remaining experimental diets in which hot cereals and similar entrees were served. Because the cereals and entrees were prepared in one receptacle, the chances of obtaining a sample which is representative of the whole is highly unlikely. This would account for the discrepancies in the "t" test results for nitrogen.

The probability that the fat and food energy will approximate the calculated values is low in all diets for fat and in five for calories (Table 10). In diets V and VII, there is a probability of 0.6 and 0.9, respectively, that the analyzed values for energy depict the values as calculated from the food tables. Since the fat revealed a low probability for all diets, it can be said that this was due to the heterogeneity of the sample. Since these diets were relatively low in fat (especially when considered on a total caloric basis), any difference in fat content would have a rather minor affect on caloric value of the total diet.

Upon making corrections for the calories according to the deviations for nitrogen and fat these results agreed very closely with the differences in calories between the calculated and analyzed figures. These

calculations were based on the assumptions that the differences were furnished by carbohydrate.

These statistical tests performed on the experimental and control diets agree with those of Patterson and McHenry (1941). These authors reported that the values for fat in the food tables may be too great. They obtained their values for calculation from the Canadian Tables of Food Composition. However, it is possible to interject the statement that the United States food composition tables are also too high for fat. If this is true, the estimated carbohydrate in the diet is too low. This is assuming that the carbohydrate is that which remains after subtracting protein, fat, and minerals. Leverton and Whiting (1961) and Widdowson and McCance (1942) reported that when the amount of variation between the calculated versus analyzed fat is greater than 10 per cent, doubt can be cast on the use of food tables for calculating fat for epidemiological studies. Furthermore, the tables should not be used with any validity for the determination of the nutrient values of diets in metabolic balance studies. Thomas et al., (1950) also found no close agreement between the calculated and analyzed fat in their studies. They state that this is due to the great variation in the concentration of fat in foods.

It is interesting to note that the analyzed mean $\pm$ standard deviation for fat in each diet is lower than the calculated values. This only further points to the fact that the composition tables are not reliable for calculating fat.

The argument for the unreliability of fat in regard to food tables must take into consideration the weighing and preparation techniques employed in the kitchen and the method used to determine fat. First are the weighing errors in the kitchen. These include weighing the

ingredients for each recipe and the amount to be served. The losses of fat during cooking produces little if any volatilization of the fats. The method of mixing the ingredients is the second source of error produced in the kitchen which affects the dispersion of the fat in the food. For this reason, Sampson et al., (1952) warned against the use of mixed dishes in a balance study. A dish consisting of many ingredients will be less accurate in total fat composition than a dish with only one or a few ingredients.

Many authors (Leichsenring et al., 1958, and Coons, 1930) did not advocate placing fat in the diet collected for analysis. This is due to the difficulty of preparing a homogeneous mixture when fatty substances are included.

In the M.S.U. study the fats were included in the diet collection to provide a measure of the total caloric intake of the subjects.

In the analytical methods there are several sources of errors. The most important errors are those in the methods itself. The fat content which was determined by the Goldfisch apparatus represents crude fat. This method involves weighing a beaker of approximately sixty grams. A one to two gram sample is extracted with diethyl ether. The fat which remains in the beaker is determined by reweighing the beaker after evaporating the solvent. This fat amounted to approximately 0.1200 grams in the diet samples analyzed. This is a minute weight in comparison to the weight of the beaker. In order to determine the reliability of this method, several samples were extracted on three occasions (Table 11).

Table 11

Per cent fat in three separate diets analyzed on three different days.

Sample	Trial[1]		
	a	b	c
1	9.13	8.92	8.91
2	9.48	9.51	9.53
3	9.67	9.74	9.78

[1] All values represent the average of duplicate analysis.

These variations appear small when employing them in determining the total fat per sample based on a dry weight basis; however, these figures give some indication of the analytical variability. The collected fat represents the fat that is ether extractable. Supposedly, the fat listed in the food tables repreeents the fat that is ether extractable. Harris (1962) reported that a more efficient and reliable method needs to be devised for determining fat. The type of fat employed in both the control and experimental regimens was butter, Wesson oil and "Blue Label Kraft" all vegetable shortening. The fat in the butter and Wesson oil was considered ether extractable. The Kraft Foods Company[2] stated that the shortening is entirely ether extractable.

Thomas et al., (1950) reported that energy values are highly subject to miscalculation. The heat of combustion factors were employed in the MSU study for comparing the calculated energy as determined by a bomb calorimeter. A non-homogeneous sample could be the factor producing the differences in the calculated versus analyzed energy. Also, if the values for fat in food tables are too high, agreement between the calculated and analyzed results could not be expected.

[2] The Kraft Foods Company (1962) Personal Communication.

The calculated nutrients in diets as reported in the literature gave a closer correlation with the analyzed values when the entire diet was analyzed rather than when the components of the same diet were analyzed individually. Hummel et al., (1942) analyzed 22 individual foods for nitrogen, fat, and calories. The diet analyzed in toto more closely approximated the calculated values than the sums of the individual components. From Table 12 similar results can be observed. In experimental diets III and IV there is essentially no difference between the values for the entire diet when the analyzed and calculated values are compared. There is also a closer agreement between the calculated values and the diet analyzed in total than when compared with the individual analyzed values in diet V. The accuracy of determining a small amount of nitrogen is one of the errors encountered in this experiment. Since the analyzed values for nitrogen are in close agreement with the calculated figures, it seems apparent that the diet should be analyzed as a total composite. Bricker et al., (1949) divided their food to be analyzed according to the type of nitrogen in the products. The results of Table 12 cast doubt on the reliability of analyzing minute amounts of food for nitrogen.

The literature does not clearly state the number of times that a diet in a balance study should be analyzed so that one can be assured that the analyzed results represent the composition of the diet. Reifenstein et al., (1945) and Bassett and Van Alstine (1935) advocated analyzing a diet several times throughout a study and thus, employing the determined results for the length of the study. It was stated by Reifenstein et al., (1945) that the most reliable

Table 12

Comparison of calculated versus analyzed values of individual foods that were used in preparing three experimental diets.

Foodstuff	E.P.[3] gm.	N. (gm.) Anal.	N. (gm.) Calc.[1]	N. (gm.) Anal.[2]
EXPERIMENTAL DIET III				
Strawberries	100	0.10	0.09	
Fruit Plate	227	0.27	0.24	
Spaghetti Sauce	200	0.40	0.32	
Shredded Cabbage/Pineapple	50	0.17	0.11	
Pettijohn	180	0.61	0.52	
Spaghetti	146	1.26	1.30	
Bread	450	6.53	7.03	
Apple Pie	130	0.31	0.52	
Refrigerator Cookies	50	0.53	0.59	
		10.18	10.72	10.7
EXPERIMENTAL DIET IV				
Orange Juice	100	0.12	0.13	
Melon Balls	75	0.08	0.07	
Vegetable Soup	250	0.25	0.25	
Creole Green Beans	170	0.34	0.49	
Lettuce	25	0.04	0.05	
Cream of Wheat	235	0.85	0.90	
Bread	475	6.91	7.44	
Molasses Cookies	50	0.44	0.49	
Ginger Cookies	75	0.80	0.82	
Cherry Kuchen	100	0.60	0.57	
		10.42	11.21	11.2

Table 12 (Cont.)

Foodstuff	E.P.[3] gm.	N. (gm.) Anal.	N. (gm.) Calc.[1]	N. (gm.) Anal.[2]
EXPERIMENTAL DIET V				
Orange Juice	100	0.12	0.13	
Fruit Salad	50	0.05	0.04	
Apricots	120	0.10	0.10	
Tomato	50	0.09	0.08	
Lettuce	50	0.08	0.10	
Cucumbers	25	0.02	0.03	
Boiled Dinner	150	0.32	0.22	
Wheatena	270	0.86	0.87	
Bread	500	7.26	7.81	
Saltine Crackers	25	0.43	0.39	
Crunchy Cookies	50	0.41	0.44	
Applesauce Cake	100	0.59	0.63	
		10.33	10.84	11.2

[1] Nitrogen represents protein content calculated from the U.S.D.A. Hdbk. No. 8 (Watt and Merrill, 1950 and 1963) divided by conversion factors for each food group as prescribed by Jones (1931) (Refer to p. 44).

[2] Represents the average of duplicate analysis of the diet collected during four successive weeks.

[3] E.P. represents edible portion of the food items.

method to be used is to analyze the diet daily or for each collection period. However, this is laborious and costly for any experiment. In this study the determined nitrogen values for the same diet prepared in successive weeks are significantly different at the 1 per cent level in all but two cases (Table 13). Diet VI shows significance at the 5 per cent level and the differences for diet IV are not significant. That is to say, in all instances except one there is significant variation in the dietary nitrogen of the same diet prepared at different times. There are two diets of the seven which show significant differences for energy; whereas, for fat all showed differences which are significant, either at the 1 or 5 per cent level. Why this occurs is difficult to understand, particularly when the diet was prepared and analyzed each week in the same manner. Of course, both diet preparation and analyses are subject to human error.

A closer agreement can be noted between the duplicate samples of all the diets than for the values secured on different days (Table 13). Thomas et al.,(1950) upon analyzing duplicate samples of a diet found the values for protein and food energy to be the most consistent with relatively small variations. However, the determined fat in the duplicate samples varied greatly. This difference as postulated by the authors was due to sampling. They further reported that kitchen techniques include inherent variability of sampling, preservation and measuring, as well as errors unavoidable in the best analytical methods. The variability of sampling in this study cannot be overlooked. It is not feasible to assume that from a kettle of mixed

Table 13

Comparison of the duplicate analyzed values of the experimental diets collected during four successive weeks.

Week	Sample[1]	Diet I	II	III	IV	V	VI	VII
		Nitrogen (Gm.)						
A	a	11.9	11.8	10.7	11.2	11.0	10.7	11.3
	b	11.9	11.8	10.9	11.0	11.0	10.7	11.3
B	a	11.8	10.5	10.4	11.1	11.2	10.6	10.5
	b	11.9	10.6	10.4	11.0	11.4	10.6	10.4
C	a	11.8	11.0	11.1	11.2	10.9	10.6	11.5
	b	11.8	11.1	11.1	11.4	10.8	10.7	11.5
D	a	11.5	11.0	10.7	11.4	11.4	11.2	11.5
	b	11.5	11.0	10.5	11.3	11.4	11.6	11.6
Significance[2]		< .01	< .01	< .01	N.S.	< .01	< .05	< .01
		Fat (Gm.)						
A	a	68.2	71.0	84.6	91.5	72.9[3]	93.3	87.6
	b	68.0	69.9	84.3	92.1	72.9[3]	93.5	87.1
B	a	78.6	79.8	72.1	82.3	73.8	84.3	69.0
	b	78.5	79.1	71.2	86.4	73.0	82.9	71.0
C	a	76.0	74.9	65.9	85.0	68.5	84.2	89.7
	b	77.0	75.6	66.2	85.3	68.5	83.6	89.9
D	a	74.2	85.0	70.1	88.7	76.3	85.9	84.2
	b	73.4	84.1	69.8	88.2	77.0	88.4	82.9
Significance[2]		< .01	< .01	< .01	< .05	< .05	< .05	< .01

Table 13 (Cont.)

Week	Sample[1]	Diet I	II	III	IV	V	VI	VII
		Food Energy (Calories)						
A	a	3282	3157	3021	3231	3087	3050	3238
	b	3289	3164	3118	3272	2911	3050	3272
B	a	3329	3084	2983	3245	3211	3115[3]	2884
	b	3322	3064	3002	3258	3190	3115[3]	2897
C	a	3256[3]	3130[3]	3097	3314	3207	3113	3405
	b	3256[3]	3130[3]	3103	3314	3194	3087	3447
D	a	3299	3167	3042	3357	3202	3137	3277
	b	3285	3161	2997	3357	3189	3170	3277
Significance[2]		< .05	< .05	< .05	< .01	N.S.	N.S.	< .01

[1] The letters a and b represent duplicate analysis of the same sample.

[2] The statistical significance was determined by one-way analysis of variance.

[3] Calculated figures due to missing data . (Snedecor, 1946)

food two identical samples can be removed. Thus when a dish of this type is prepared at different intervals there is less possibility that the samples removed will be the same in composition. Thus in a study, such as the "bread study", where many mixed dishes and desserts are required, the diet must be analyzed each day to assure the exact content of the diets.

Coons (1930) advocated that the diet sample should be collected for analysis at the time it is served. The serving period of a non-clinical balance study cannot be accurately controlled. The subjects in this study were informed to come within a two hour period, but due to examinations, seminars, and other committments on campus, there was not complete adherence to this policy. A time schedule, devised to aid this problem, resulted in only a slight improvement.

The results of the sampling time for the control period are portrayed in Table 14. The means of both serving times show only slight differences in the smount of nitrogen, fat, or food energy analyzed in the diets. The "t" test reveals that there is no difference between the samples collected at zero hour and one and one-half hour. There is a high probability for each nutrient that the analyzed values will be in close proximity regardless of the time it is collected.

The results for the experimental diet (Table 15) agree with the control diet for food energy and fat, but for nitrogen a difference with time is demonstrated. The standard deviation for nitrogen is small which indicates that the nitrogen from diet to diet had small variation. This factor is supported by the results of the 3-way analysis of variance as mentioned previously.

Table 14

The analyzed values for nitrogen, fat and calories
for the control diets collected at two time intervals during the serving period.

DIET	Nitrogen (gm.)			Fat (gm.)			Food Energy (calories)		
	Time of Collection			Time of Collection			Time of Collection		
	0-Hour	1½-Hour	Δ	0-Hour	1½-Hour	Δ	0-Hour	1½-Hour	Δ
I	11.5	11.3	-0.2	110.5	105.3	-5.2	3100	3070	- 30
II	13.2	12.7	-0.5	125.2	125.1	-0.1	3256	3224	- 28
III	10.7	10.8	+0.1	105.3	104.8	-0.5	2860	2852	- 8
IV	11.2	10.9	-0.3	97.2	93.0	-4.2	3112	2949	-163
V	10.6	10.8	+0.2	91.7	95.0	+3.3	3012	2941	- 71
VI	11.1	11.3	+0.2	90.2	92.3	+2.1	2730	2814	+ 84
VII	11.5	11.4	-0.1	94.8	101.8	+7.0	2904	2936	+ 32
Mean	11.4	11.3	0.2	102.1	102.5	3.2	2996	2969	59
Delta Mean	0.43	0.41	0.11	9.9	7.9	2.0	141	101	36
S.D.	0.57	0.65	0.16	12.5	11.4	2.5	178	139	51

$t = 0.218$ $P = >.8$ | $t = 0.058$ $P = > .9$ | $t = 0.283$ $P = < .8$

Table 15

The analyzed values for nitrogen, fat and calories
for the experimental diets collected at two time intervals during the serving period.

DIET	Nitrogen (gm.)			Fat (gm.)			Food Energy (calories)		
	Time of Collection			Time of Collection			Time of Collection		
	0-Hour	1½-Hour	Δ	0-Hour	1½-Hour	Δ	0-Hour	1½-Hour	Δ
ID	11.5	11.7	0.2	73.9	69.6	-4.3	3292	3158	-134
IID	11.0	11.4	0.4	84.6	80.7	-3.9	3161	3221	60
IIID	10.6	11.1	0.5	70.1	83.8	13.7	3023	3246	223
IVD	11.2	11.4	0.2	88.4	89.6	1.2	3357	3353	- 4
VD	11.4	11.6	0.2	76.7	78.9	2.2	3196	3308	112
VID	11.4	11.3	-0.1	87.2	93.9	6.7	3157	3001	-156
VIID	11.6	11.5	-0.1	83.6	81.2	-2.4	3277	3333	56
Mean	11.2	11.4	0.2	80.6	82.5	4.9	3209	3231	106
Delta Mean	0.3	0.1	0.12	6.1	5.6	3.0	85	96	57
S.D.	0.4	0.2	0.15	7.1	7.8	4.3	110	125	73
	t = 1.20			t = 0.438			t = 0.260		
	P = <.3			P = <.7			P = <.8		

The results reveal that the nitrogen values for one and one-half hour are greater in five of the seven diets than the values analyzed for zero hour. Diets VI and VII show slight trends in the opposite direction. In viewing the constitutents which composed each diet (Tables 4 and 5) it can be observed that both menus, control and experimental, contained mixed entrees and desserts. Fewer mixed entrees were employed in the control regimen. Diet VI contained a mixed dish entree for which the individual servings were weighed prior to cooking. This was performed with pizza. This would produce a greater uniformity between the individual servings. Macaroni salad in diet VI was mixed in one dish and then the individual portions were weighed. However, this had only an infinitesimal amount of liquid. Thus, it can be said that because of the compactness of this dish, a serving was likely to represent the entire salad. Many of the entrees in the diet contained a fair amount of liquid. This would produce more error in obtaining samples which are representative of the whole dish. Because of the dryness of the bread that was required during the experimental period the liquid dishes made the meals more palatable. This was borne out by the fact that these meals were easier to consume.

Experimental diets I through V all contained hot cereal. It can be postulated that there was a loss in moisture in the cereal upon standing; thus the last serving was more concentrated in nitrogen. The control diets contained hot cereal only in diet II. Table 14 shows that for nitrogen in control diet II there is the greatest change between the two collection periods. However, this change is opposite to the results revealed in the experimental diets. This deviation in the results could be attributed to the bread dressing

served at dinner. Since two duplicate samples do not contain the same components, the serving at one and one-half hour might have contained less nitrogen than the sample at zero hour. Where mixed entrees or cooked cereals are served from one receptacle, there are possible changes in the nitrogen composition as the serving time is extended. Because of the way the cereals were prepared, it is inevitable that there was a moisture loss with time. Since cereals contributed notrogen to the experimental diets in a fairly large amount, they should have been prepared on an individual basis. This method (Presson, 1955) gives a high degree of accuracy. Unfortunately, this could not have been done in the kitchen employed in the "bread study".

The analyzed values for nitrogen show a closer agreement than the determined fat values in the individual meals (Table 16) collected at different times during the meal service. It can be assumed that if the values are within 10 per cent of the maximum deviation there is no difference in the values with regard to sampling time.

The specific sampling time varies with the objectives of the balance study. It is apparent that when the entire diet is sampled nitrogen is the constituent which varies most over the one and one-half hour collection period of the experimental regimen. The analyzed nitrogen in the individual meals disclose only small differences. Upon analysis for fat in the individual meals the maximum variations from the mean range from 5.7 to 39.4 per cent. This is in agreement with the fat values discussed throughout this presentation. This great deviation in fat is due to the difficulty of preparing and obtaining food samples which are homogeneous in their fat content.

Table 16

Analysis of individual meals collected at different serving time.

Collection Time	Nitrogen (Gm.)			Fat (Gm.)		
	Breakfast	Lunch	Dinner	Breakfast[1]	Lunch	Dinner
	Experimental Diet IA					
0-Hour	2.3	3.6	5.9	31.4	13.8	18.7
½-Hour	2.4	3.7	5.8	35.9	11.7	17.6
1-Hour	2.4	3.7	5.7	35.3	12.7	12.4
1½-Hour	2.4	3.8	5.9	35.0	12.0	15.3
Mean	2.37	3.7	5.8	34.4	12.6	16.0
Max. Difference	0.1	0.2	0.2	4.5	2.1	6.3
Max. Deviation as % of mean	4.2	5.4	3.4	13.1	16.7	39.4
	Experimental Diet IIA					
0-Hour	2.8	3.5	5.2	36.3	9.5	27.0
½-Hour	2.8	3.6	5.2	32.0	10.9	26.4
1-Hour	2.9	3.7	5.4	36.3	12.2	25.5
1½-Hour	2.8	3.6	5.1	29.7	11.0	26.4
Mean	2.85	3.6	5.2	33.6	10.9	26.3
Max. Difference	0.1	0.2	0.3	6.6	2.7	1.5
Max. Deviation as % of mean	3.9	5.6	5.8	19.6	24.8	5.7

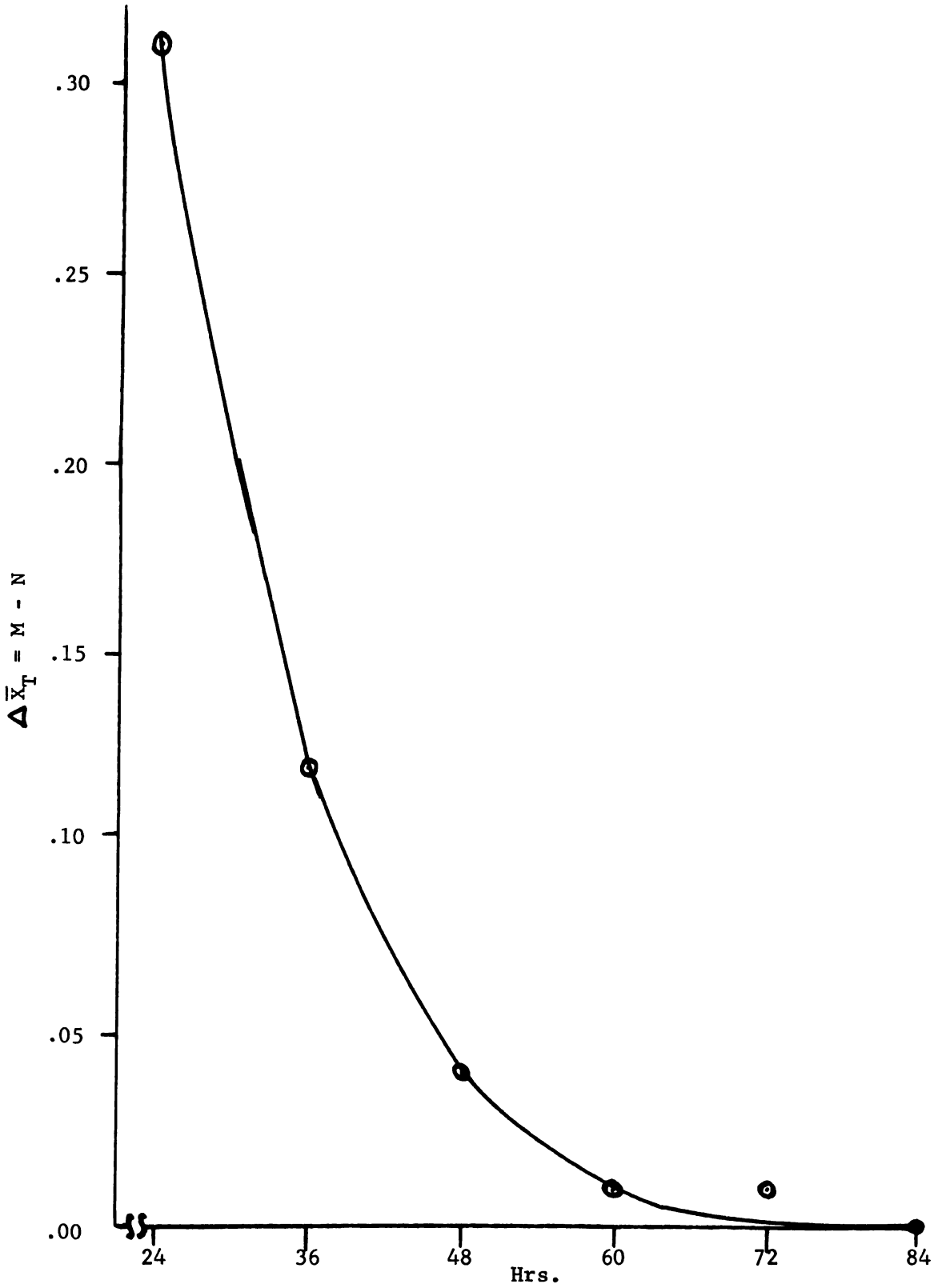
[1] Butter allotted for day was included in the breakfast samples.

Probably the diet sample for analysis should be collected when the majority of the subjects are being served in order to be relatively assured that the analyzed nutrients in the diet represent the diets consumed by the subjects.

In the Spring of 1964, the diets were dried in an oven at 60° C. for twenty-four hours using a vacuum of 28 inches of Hg. In the Spring of 1965, the same procedure was used to dry the diets except that they were dried until constant weights were obtained. From Figure 1 it can be observed that after 60 hours of drying time there is no appreciable change in moisture content. Thus it is accurate to consider the sample dry after 60 hours of drying when using the above specified conditions. Table 17 presents the per cent of moisture obtained when the experimental diets were analyzed on two separate occasions. A loss of moisture upon storage is shown for all of the diet samples. A mean loss of 3.95 ± 3.53 per cent occurs in the experimental diets.

To further evaluate the moisture loss upon storage, two cartons were filled with a measured amount of distilled water. This experiment showed a 7.5 and a 10.0 per cent moisture loss after being stored in a freezer for a two month and a three and one-half month period, respectively. The loss of moisture was measured volumetrically and metrically. Since water was used, a greater loss is disclosed than is seen in Table 17. This is due to the fact that the water is maintained in the diet suspension and, therefore, less evaporation can occur. This moisture loss could be attributed to several factors. First, the cover did not fit tightly on all the cartons. Second, it is possible that some of the moisture was absorbed into the walls of

Figure 1. Effects on drying with time.



Where M is the average moisture (in %) of 33 exptl. diets dried in duplicate for the hrs. indicated on the abscissa and N is the average moisture (in%) of the same samples after they were dried to constant weight.

Table 17

Moisture Determinations on diet samples.

Exper. Diet	% Moisture[1] Spring, 1964	% Moisture[2] Spring, 1965	Δ	Δ as % of Spring, 1965
IA_1	N.A.[3]	70.04	--	--
IA_4	N.A.[3]	69.97	--	--
IB	70.21	69.35	0.86	1.24
IC	72.70	71.10	1.60	2.25
ID_1	70.43	69.61	0.82	1.18
ID_4	70.19	68.32	1.87	2.74
IIA_1	N.A.[3]	71.28	--	--
IIA_4	N. A.[3]	71.54	--	--
IIB	72.42	70.46	1.96	2.78
IIC	72.62	69.84	2.78	3.98
IID_1	71.54	70.82	0.72	1.02
IID_4	70.80	68.68	2.12	3.09
IIIA	72.05	71.94	0.11	0.15
IIIB	72.42	66.31	6.11	9.21
IIIC	71.19	69.02	2.17	3.14
$IIID_1$	72.04	67.72	4.32	6.38
$IIID_4$	70.09	68.10	1.99	2.92
IVA	71.51	71.19	0.32	0.45
IVB	70.86	68.34	2.52	3.69
IVC	70.12	67.32	2.80	4.16
IVD_1	70.07	68.98	1.09	1.58
IVD_4	69.58	69.20	0.38	0.55
VA	73.48	73.42	0.06	0.08
VB	69.98	66.76	3.22	4.82
VC	70.15	65.59	4.56	6.95
VD_1	70.12	68.70	1.42	2.07
VD_4	68.56	67.16	1.40	2.08
VIA	72.38	71.94	0.44	0.61
VIB	74.66	70.92	3.74	5.27
VIC	71.58	66.34	5.24	7.90
VID_1	71.25	68.08	3.17	4.66
VID_4	71.59	67.54	4.05	6.00
VIIA	68.15	67.58	0.57	0.84
VIIB	69.50	60.14	9.36	15.56
VIIC	66.29	62.20	4.09	6.58
$VIID_1$	67.20	59.29	7.91	13.34
$VIID_4$	66.76	64.67	2.09	3.23

Mean Per Cent = 3.95%

Delta Mean Per Cent = 2.63%

S.D. = 3.53%

Table 17 (Cont.)

[1] Average of duplicate samples dried for 24 hours at 60°C in a vacuum oven.

[2] Average of duplicate samples dried at 60°C in a vacuum oven until constant weight was obtained.

[3] Diets not analyzed Spring, 1964.

Subscript 1 represents collection at zero hour.
Subscript 4 represents collection at one and one-half hour.

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the carton. Third, some moisture loss could occur when the sample is removed for analysis from the same carton at different times since this involves thawing and refreezing the samples.

The literature does not discuss moisture losses due to storage. The type of container and the amount of sample placed in each container should be emphasized. The container employed to store the diet sample should be air tight and non-porous. Furthermore, to avoid losses in moisture which occur upon thawing and refreezing the sample, the amount to be used at one time for analysis should be stored in individual containers. Whenever more samples are required for analysis, a sample which has not been opened previously could be removed from the freezer, thawed, and dried.

SUMMARY AND CONCLUSIONS

Twelve subjects participated in a study to determine whether or not they could remain in positive nitrogen equilibrium when consuming a diet which contained 90 to 95 per cent of their protein intake from wheat products. This study was conducted in a non-clinical situation. From this study various conclusions can be made concerning the conduct and dietary management of such a study.

The selection of the subjects should be based on their age, sex, health, dietary habits, integrity, and dependability. The health status of the subjects should be evaluated by a medical physician. Because this is such a broad statement, specific instructions for the type of evaluation desired should be given to the physician. Persons with physical handicaps or not within 10 per cent of their ideal weight should be eliminated in studies which attempt to evaluate normal individuals. To eliminate those subjects who may have emotional problems to the extent that they would be unreliable, irresponsible, and dishonest, a psychological examination evaluating this aspect should be given.

To acquaint the personnel and subjects with the demands of the study a trial period should be included. This trial period preferably should be a part of the control period. It is during this time that the subjects and personnel become adapted to the controls and procedures of the study.

Because the subjects were going to consume a low protein diet during the experimental period, the control period provided a diet which contained the same amount of amino acids as would be provided by

the experimental diet. This was done to permit the subjects to establish nitrogen equilibrium prior to the initiation of the experimental diet. For this reason no transitional period was required between the control and experimental periods.

For psychological and emotional reasons rapport between the researchers and the subjects must be established very soon after the study begins. One factor that aids in this aspect is a "homey" and pleasant atmosphere in which the subjects eat their meals.

The purchase and selection of food items is also a very important facet in the conduct of any metabolic balance study. All food used must be of the same brand and from the same lot. To purchase all the food at the start of the study requires adequate and proper storage facilities.

The type of menus used are limited by the purpose of the metabolic study. Since this study was concerned with nitrogen balance, a large variety of food items and methods of preparation could be used. If the purpose of this study was to accurately determine fat, food energy, and various other nutrients, mixed dishes certainly could not be used.

Coffee, tea, and sugar were allowed ad libitum. Since the first two items contained nitrogen, the amount consumed by the subjects must be known. The subjects were required to record all of these items which they consumed. Ideally, instant coffee and tea should be provided in weighed packages that contain approximately enough for one balance period. The amount consumed should be measured from the weight of this material remaining at the end of each balance period.

If the subjects are of "normal" weight and the intention of the study is to maintain such a body weight, proper precautions should be taken to accomplish it. For subjects who were losing weight non-protein foods such as sugar, honey, hard candies, jelly, and whey-free butter were administered. Those individuals gaining weight were restricted in their use of whey-free butter, jelly, and sugar.

The weight loss which was observed in the majority of the subjects during the experimental regimen was hypothesized to be due to the increased physical activity which was brought about with advent of summer-like weather. Therefore, if the purpose of a balance study is to maintain the weights of the subjects, it should occur during the time of year when there is the least change in weather conditions.

For a metabolic balance study of this nature the Hanson spring scale and the Toledo scale are sufficiently accurate providing they are checked at frequent intervals during the study.

To determine the amount of protein, fat, carbohydrate, and other essential nutrients in the diets tables of food composition must be employed. However, complete confidence in such tables is not valid as was shown from the comparisons of the analyzed values of the diets with the calculated values of the same diets.

The factors used to convert the calculated protein to nitrogen and the calculated carbohydrate, fat, and protein to food energy are numerous and must be used with discretion. Jones' factors used to calculate the nitrogen from the calculated protein values were: milk,6.38; meat, eggs, fish, 6.25; wheat and cereals, 5.70; and nuts and seeds, 5.30. These factors are more specific for each respective

food group than is 6.25. The factors used to convert grams of carbohydrate, fat, and protein to calories were 4, 9, 4, respectively, and the specific heat of combustion factors for each food group. Heat of combustion factors must be used if a comparison is to be made with the determined values obtained from an oxygen bomb calorimeter.

There is no statistically significant difference between the calculated and analyzed values for nitrogen in the experimental diets as shown by the 3-way analysis of variance test. The values for fat and calories were significantly different. The levels of significance for fat and food energy are one per cent and five per cent, respectively. All of the analyzed values for fat were lower than the calculated values. Five of the seven control diets showed a deviation greater than 10 per cent. Upon comparing the calculated versus analyzed values for fat in the experimental diets 50 per cent showed a deviation greater than 10 per cent. All but two of the analyzed values for food energy in the control diets were lower than the calculated values. Two of these diets showed a deviation greater than 10 per cent. Eight out of the twenty-eight experimental diets had larger calculated values than the analyzed values for food energy. Only one of the experimental diets showed a deviation greater than 10 per cent. In comparing the calculated versus analyzed values for fat and calories there were fewer diets in this study that had a deviation greater than 10 per cent when compared to the values presented in the literature .

A closer correlation is observed when the diet is analyzed <u>in toto</u> and compared with the calculated values than when the individual components of the same diet are analyzed and summed.

Various reports state that analysis of diets needs to be performed only occasionally and the results obtained can be used as the actual

composition of the diet throughout a balance study. Analyses performed on diets replicated during four successive weeks showed that it is important to analyze the diets each time the diet is prepared.

No significance was observed between the chemically determined nitrogen, fat, or calories when the meals were collected at two different times during the meal serving period. However, for the nitrogen in the experimental diets the "t" test showed that the analysis does depend upon time. Contrarily, when individual meals of the experimental diets were collected at four intervals during the meal service, no significant differences were observed for nitrogen. There was a significant difference for fat. Therefore, it appears that the analyses of the diets does depend somewhat upon the time the diet sample is collected. The most accurate time is probably when most of the subjects are being served.

It appears that the drying time required to completely dry a diet sample is sixty hours using a temperature of 60^{o} C in an oven with a vacuum of 28 inches of Hg.

The container used to store the diet samples for analyses should be non-porous. Because moisture loss occurs when a sample is frozen and rethawed, only enough sample which can be used for the determinations on one occasion should be stored in a container. The most suitable container is a glass jar with a tight fitting cover or lid.

Good results can be obtained from a non-clinical metabolic balance study if the proper precautions and techniques are employed.

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APPENDIX

THE RECIPES

Special recipes were devised for the "bread study" in order to fulfill the requirement for the type of protein in the diets. These recipes were formulated with the aid of the following references:

1. "Better Homes and Gardens New Cook Book." 1953. 1st ed., Meredith Publishing Co., New York.
2. "Betty Crocker's Picture Cook Book." 1956. 2nd ed., McGraw Hill Book Co., New York.
3. Farmer, F. M. 1951. "The Boston Cooking-School Cook Book." 9th ed., Little Brown and Co., Boston.
4. Fowler, S. and B. West. 1950 "Food for Fifty." John Wiley and Sons, Inc., New York.
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The recipes which follow are listed in alphabetical order.

Apple Pie

Yield: Two 9" pies

Temp.: 325° F

Time : 55 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Apples (sliced, water packed)	1550	170.5	3.4	1.3
Sugar	300	300.0	---	---
Cinnamon	4	---	---	---
Butter (protein free)	30	---	---	24.0
Pie Crust (raw weight)	842	324.8	56.1	274.4
Prepared Weight:	2606	795.3	59.5	299.7
Serving: (one)	130	39.7	3.0	15.0

Instructions: Make double pie crust (see pie crust). Weigh 421 grams of crust for each pie. Use approximately 210 grams for each crust. Roll out crusts between two sheets of wax paper. Slit pastry for top crusts in several places. Place bottom crust in 9" pie pan. Place 775 grams apples on bottom crust. Combine 150 grams of sugar and 2 grams of cinnamon. Sprinkle over apples. Dot with 15 grams butter. Cover with top crust. Seal edges securely.

Applesauce Cake

Yield: Four 8" x 8" cakes

Temp.: 375°F

Time : 20 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Flour	400	295.2	51.0	4.0
Baking soda	7	---	---	---
Salt	8	---	---	---
Cinnamon	11	---	---	---
Cloves	2	---	---	---
Allspice	7	---	---	---
Nutmeg	7	---	---	---
Shortening	230	---	---	230.0
Sugar	460	460.0	---	---
Applesauce (canned, sweetened)	550	114.6	1.4	0.9
Prepared weight:	1453	869.8	52.4	234.9
Serving (one) :	100	59.9	3.6	16.2

Instructions: Line four 8" x 8" pans with wax paper. Weigh and combine flour, baking soda, salt, cinnamon, cloves, allspice and nutmeg. Cream shortening and sugar until light and fluffy in electric mixer bowl. Add sifted dry ingredients alternately with the applesauce, beating well after each addition. Pour 420 grams into a wax paper lined 8" x 8" pan.

Boiled Dinner

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Carrots (canned, drained)	670	42.9	4.5	3.6
Onions (raw, diced)	110	11.3	1.5	0.2
Potatoes (raw, diced)	700	133.7	14.0	0.7
Cabbage (raw, shredded)	340	18.4	4.8	0.7
Celery (raw, diced)	300	11.1	3.9	0.6
Vegetable bouillon cubes (1 cube)	4	—	0.5	—
Water	1500	—	—	—
Prepared Weight:	3234	217.4	29.2	5.8
Serving (one) :	150	10.1	1.4	0.3

Instructions: Cook raw vegetables in weighed amount of water with vegetable bouillon cube in 8 quart aluminum kettle. When done add carrots. Heat thoroughly. Serve 150 grams in individual casserole dishes. Cover and place in warming oven.

Bread

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Flour	4790	3535.5	611.2	47.9
Shortening	200[1]	---	---	200.0
Sugar	150	150.0	---	---
Salt	85	---	---	---
Yeast, dry (7 pkg.)	49	19.3	18.3	0.8
Water	2880	---	---	---
Prepared Weight:	7047	3704.8	629.5	248.7
Serving per 100:		52.6	8.9	3.5

Instructions: Place into a 2 quart mixing bowl yeast, 6 teaspoons of weighed sugar and 360 grams of lukewarm water. Water should be 43°C. Please test with thermometer. Set bowl in proofing area. Weigh 2520 grams of water and pour in a large Hobart mixing bowl. Add 150 grams shortening, remaining sugar and 85 grams salt. Mix 2 minutes at low speed. Add 10 cups of flour. Mix 3 minutes at low speed. At this time be sure dough is lukewarm (43°C).

If dough is correct temperature, add yeast mixture. Add the remaining flour very slowly. Be sure to stop mixer for each addition. After all flour and ingredients are mixed, knead at low speed for 14 minutes.

Grease sides of mixing bowl and top of dough lightly with shortening allowed for handling dough. Cover dough with wax paper and a cloth towel. Place in proofing area. Mark time.

[1] 150 grams in recipe and 50 grams for handling dough.

Let rise 1½ hours. Punch down and knead at low speed for 2 minutes. Brush top of dough with shortening as above. Cover and return to proofing area for 45 minutes. Mark time. While dough is rising --

Grease lightly 12 bread pans from the shortening allowed for handling.[2]

Weigh approximately 500 grams of dough and form into a loaf. Place in greased pans. Cover as above. Return to proofing area. Mark time. Let rise 1 hour. Preheat oven 350°F. Bake 35 minutes.

[2] For rolls, weigh the amount requested and use 50 grams for each roll. Place in greased 9" x 13" pan. Bake 20 minutes at 425°F.

For French bread, shape 500 grams dough into oblong loaf. Place on greased cooking sheet. Bake at 350°F for 35 minutes.

Bread Dressing

Temp.: 350°F

Time : 60 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Bread Crumbs	840	441.6	75.0	2.9
Butter, melted	175	1.4	1.5	140.9
Salt	17	---	---	---
Pepper	2	---	---	---
Onion, minced	70	7.0	0.7	---
Celery, minced	350	13.0	4.6	0.7
Water	1850	---	---	---
Prepared Weight:	2643	463.0	81.8	144.5
Serving (one) :	150	26.3	4.6	8.2

Instructions: Combine all ingredients except water. Mix thoroughly. Add water and mix. Place in 9" x 13" pan and bake.

Carrot/Pineapple Salad

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Carrots (raw)	420	39.1	5.0	1.3
Pineapple slices (sirup packed)	280	59.0	1.2	0.2
Prepared Weight:	700	98.1	6.2	1.5
Serving (one):	50	7.0	0.4	0.1

Instructions: Grate carrots. Drain pineapple. Cut in chunks. Stir in pineapple. Weigh 50 grams for each subject.

Cherry Kuchen (Control)

Yield: Three 8" x 8" Kuchens

Temp.: 350°F

Time : 45 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Water	270	---	---	---
Sugar	450	450.0	---	---
Flour [1]	450	---	---	---
Baking Powder	14	---	---	---
Salt	11	---	---	---
Water	270	---	---	---
Butter (melted) [2, 3]	280	---	---	---
Vanilla	12	---	---	---
Topping:				
Flour [1]	85	---	---	---
Brown Sugar	110	105.0	---	---
Butter [2]	56	---	---	---
Baking Powder	4	---	---	---
Cherries (water packed, drained)	460	47.6	3.2	1.2
[1] Total Flour Values	535	394.9	68.3	5.3
[2] Total Butter Values	336	2.7	1.9	270.0
Prepared Weight:	2233	1000.2	73.4	276.5
Serving (one) :	100	44.8	3.3	12.4

[3] 15 grams of butter is to be employed for lightly greasing the pans.

Instructions: Lightly grease three 8" x 8" pans with the allotted butter. Blend 270 grams water and sugar. Add sifted flour, baking powder, and salt. Add remaining water, melted butter, and vanilla. Combine and mix all topping ingredients. Into each pan place 500 grams of batter, 130 grams of drained cherries, and sprinkle with 70 grams of topping.

Cherry Kuchen (Experimental)

Yield: Three 8" x 8" Kuchens

Temp.: 350°F

Time : 45 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Water	270	---	---	---
Sugar	450	450.0	---	---
Flour [1]	450	---	---	---
Baking Powder	14	---	---	---
Salt	11	---	---	---
Water	270	---	---	---
Butter, melted [2, 3] (protein free)	280	---	---	---
Vanilla	12	---	---	---
Topping:				
Flour [1]	85	---	---	---
Brown Sugar	110	105.0	---	---
Butter [2]	56	---	---	---
Baking Powder	4	---	---	---
Cherries (water packed, drained)	460	47.6	3.2	1.2
[1] Total Flour Value	535	394.9	68.3	5.3
[2] Total Butter Value	336	---	---	268.8
[3] 15 grams of butter is to be employed for lightly greasing pan.				
Prepared Weight:	2188	997.5	71.5	275.3
Serving (one) :	100	45.6	3.3	12.6

Instructions: See Control.

Cherry Pie (Control)

Yield: Two 9" pies

Temp. and Time: 425°F for 10 minutes
350°F for 40 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Filling:				
Cherries (water packed, drained)	760	78.6	5.3	2.0
Sugar	300	300.0	---	---
Flour	56	41.3	7.2	0.5
Juice from cherries	240	trace	trace	trace
Butter	56	0.4	0.3	45.1
Pie Crust	840	324.8	56.1	274.4
Prepared Weight: (Filling & Crust)	2038	745.2	68.9	322.0
Serving (one) :	135	49.4	4.6	21.3

Instructions: Make double pie crust (See pie crust). Weigh 421 grams of crust for each pie. Use approximately 210 grams for each crust. Roll out crusts between two sheets of wax paper. Slit pastry for top crust in several places. Place bottom crust in 9" pie pan. Drain cherries. Save juice. Mix sugar and flour in 3 quart sauce pan. Add 240 grams of the juice. Bring to boiling point. Remove and stir in drained cherries. Fill each pie crust with 756 grams of filling.

Cherry Pie (Experimental)

Yield: Two 9" pies

Temp. and Time: 425°F for 10 minutes

350°F for 40 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Filling:				
Cherries (water packed, drained)	760	78.6	5.3	2.0
Sugar	300	300.0	---	---
Flour	56	41.3	7.2	0.5
Juice from cherries	240	trace	trace	trace
Butter (protein-free)	56	---	---	44.8
Pie Crust	840	324.8	56.1	274.4
Prepared Weight: (Crust & Filling)	2087	744.7	68.6	321.7
Serving (one):	130	46.4	4.3	20.0

Instructions: See Cherry Pie (Control).

Chocolate Cake

Yield: Four 8" x 8" layers

Temp.: 375°F

Time : 30 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Shortening	200	---	---	200.0
Vanilla	8	---	---	---
Sugar	500	500.0	---	---
Chocolate Squares	100	29.2	5.5	52.9
Flour	400	295.2	51.0	4.0
Salt	5	---	---	---
Baking Powder	16	---	---	---
Water	480	---	---	---
Prepared Weight:	1482	824.4	56.5	256.9
Serving (one) :	100	55.6	3.8	17.3

Instructions: Line four 8"x 8" cake pans with wax paper. Grate chocolate. Cream shortening, add vanilla and beat in (by hand) sugar and grated chocolate. Combine flour with salt and baking powder. Add flour mixture alternately with water to shortening mixture. Pour 428 grams into each 8"x 8" layer pan.

Cream of Wheat

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Water	3636	---	---	---
Salt	10.	---	---	---
Cream of Wheat (Quick)	684	1022.2	79.2	7.2
Prepared Weight:	3643	1022.2	79.2	7.2
Serving (one) :	235	65.9	5.1	0.5
Water	2376	---	---	---
Salt	8.	---	---	---
Cream of Wheat (Quick)	456	681.4	52.8	4.8
Prepared Weight:	2387	681.4	52.8	4.8
Serving (one) :	177	50.5	3.9	0.4

Instructions: Bring water and salt to rapid boil. Slowly sprinkle in Cream of Wheat stirring constantly while the mixture thickens. Lower heat and cook 5 minutes. Place over pan of hot water to keep warm for serving.

Creole Green Beans

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Onion (raw, chopped)	50	10.3	1.4	0.2
Salad oil	60	---	---	60.0
Chili Sauce	360	178.6	18.0	2.2
Salt	13	---	---	---
Green Beans (canned, drained)	1200	122.9	37.5	4.1
Prepared Weight:	3132	311.8	56.9	66.5
Serving (one) :	170	16.9	3.1	3.6

Instructions: Brown onion in oil in 8 quart aluminum kettle. Add chili sauce. Add green beans. Heat thoroughly. Place 170 grams in individual casseroles. Place in warming oven until served.

Creole Spaghetti Sauce

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Sauce:				
Onion (raw, minced)	160	16.0	1.6	---
Green Pepper (raw, chopped)	160	9.2	1.6	0.4
Vegetable Oil	56	---	---	56.0
Ground Beef (cooked weight)	200	---	51.3	34.1
Salt	15	---	---	---
Sugar	6	6.0	---	---
Tomatoes (canned) (juice and solids)	1700	66.3	17.0	3.4
Prepared Weight:	2068	97.5	71.5	93.9
Serving (one) :	100	4.7	3.4	4.5

Instructions: Cook ground beef in frying pan. Brown onion and green pepper in oil in aluminum 8 quart kettle. Stir in ground beef and any fat from beef, salt, sugar, and tomatoes. Simmer for 30 minutes. Pour 100 grams of sauce over 100 grams cooked spaghetti in individual casserole dishes. Cover with casserole lid and place in warming oven.

Crunchy Cookies

Temp.: 350°F

Time : 10 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Shortening	400	---	---	400.0
Brown Sugar	440	420.0	---	---
White Sugar	400	400.0	---	---
Water	120	---	---	---
Vanilla	15	---	---	---
Salt	8	---	---	---
Baking Powder	10	---	---	---
Flour	600	442.9	76.6	6.0
Wheaties	260	208.9	26.0	5.6
Prepared Weight:	2049	1471.8	102.6	411.6
Serving (one) :	25	17.9	1.2	5.0

Instructions: Thoroughly cream shortening and sugars in Hobart mixing bowl. Add water and vanilla. Beat well. Combine dry ingredients. Add to previous mixture. Add wheaties. Mix 1 minute(low speed). Bake on ungreased cooky sheet. Remove from baking sheet and place on wax paper. When cool, weigh and store in plastic bags in the walk-in refrigerator. Be sure to label the bag with the contents, weight, and the date prepared.

Cupcakes

Yield: 18 cupcakes

Temp.: 375°F

Time : 25 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Shortening	134	---	---	134.0
Flour	400	295.2	51.0	4.0
Sugar	400	400.0	---	---
Baking Powder	12	---	---	---
Salt	7	---	---	---
Milk	365	18.6	13.5	12.7
Egg (Approximately 2)	108	0.6	13.8	11.0
Vanilla	8	---	---	---
Prepared Weight:	1130	714.4	78.3	161.7
Serving (one) :	50	31.6	3.5	7.2

Instructions: Mix shortening to soften in electric mixer bowl. Sift in dry ingredients. Add one-half of milk and the eggs. Mix only to dampen flour mixture. Add remaining milk and vanilla. Beat for one minute at low speed. Line the muffin tin with the paper muffin cups. Fill each with 60 grams of dough.

Dumplings

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Flour	990	730.7	126.3	9.9
Baking Powder	53	---	---	---
Salt	15	---	---	---
Butter (Protein free)	60	---	---	48.0
Water	810	---	---	---
Prepared Weight:	1939	730.7	126.3	57.9
Serving (one) :	120 [1]	45.2	7.8	3.6

Instructions: Combine all ingredients and mix well. Weigh out 120 grams raw weight per subject. Place the dumpling in the vegetarian stew. Cook 20 minutes.

Farina

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Farina	608	473.6	60.8	4.8
Water	3232	---	---	---
Salt	10	---	---	---
Prepared Weight:	3500	473.6	60.8	4.8
Serving (one) :	250	33.8	4.3	0.3

Instructions: Bring water to vigorous boil in 8 quart aluminum kettle. Add salt, then add Farina very slowly (to avoid lumping), stirring constantly. Return to boiling point. Lower heat half-way and cook 2½ minutes stirring frequently. Place pan with cooked cereal in receptacle of hot water to keep warm for serving.

[1] 120 grams wet weight - yielded 150 grams cooked dumpling.

Fruit Ice

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Water	300	--	--	--
Sugar	400	400.0	--	--
Salt	2	--	--	--
Orange Juice (frozen, diluted 1:3)	240	26.2	1.9	--
Lemon Juice (Real Lemon)	160	12.3	0.6	0.3
Banana (fresh)	450	103.5	5.4	0.9
Prepared Weight:	1505	542.0	7.9	1.2
Serving: (one)	100	36.0	0.5	0.1

Instructions: Place bananas in electric mixing bowl: mix at low speed. Add juices, sugar, and water to the banana. Mix well. Pour into ice cube trays. Place in freezer of refrigerator. Freeze 24 hours before serving.

Fruit Plate (Individual Plate)

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Pear Half	50	9.2	0.1	---
Pineapple Slices[1]	65	---	---	---
Banana	75	17.2	0.9	0.2
Pineapple Juice[1]	15	---	---	---
Lettuce	20	0.6	0.2	---
Maraschino Cherry	2	0.6	---	---
[1] Total values for pineapple	80	16.9	0.3	0.1
Prepared Weight:	227	44.5	1.5	0.3
Serving (one) :	227	44.5	1.5	0.3

Instructions: Drain all fruit before weighing. Arrange fruit on lettuce. Pineapple juice is to be placed over the banana to avoid darkening.

Fruit Salad

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Pineapple slices (canned)	200	42.2	0.8	0.2
Banana (slices)	250	46.0	2.4	0.4
Apples (unpeeled, cubed)	250	27.5	0.5	0.2
Prepared Weight:	700	115.7	3.7	0.8
Serving (one):	50	8.2	0.3	0.1

Instructions: Drain pineapple and cut slices into dices. Prepare bananas and apples as stated above. Toss all fruits together. Serve 50 grams on 20 grams of lettuce to each subject.

Ginger Cookies

Temp.: 375° F

Time : 15 minutes

Ingredient	Gram weight	CHO gm.	Protein gm.	Fat gm.
Shortening[1]	225	--	--	225.0
Sugar	225	225.0	--	--
Molasses (medium)	370	240.5	--	--
Vinegar	20	--	--	--
Flour	745	549.9	95.1	7.4
Soda	3	--	--	--
Cinnamon	3	--	--	--
Ginger	3	--	--	--
Salt	3	--	--	--
Water	125	--	--	--
Prepared Weight:	1517	1015.4	95.1	232.4
Serving: (one)	25	16.7	1.6	3.8

Instructions: Bring shortening, sugar, molasses, vinegar to a boil in 8-quart aluminum kettle. Cool. Add water. Combine dry ingredients. Add to liquid ingredients and mix well. Shape in a roll, wrap in wax paper and chill. Cut into slices and bake on lightly greased cookie sheet at 375° for 15 minutes. Remove from baking sheet and place on wax paper. When cool, weigh and store in plastic bags in the refrigerated walk-in. Be sure to label the bag with the contents, weight and the date prepared.

[1]ten grams of the shortening is to be employed to lightly grease the baking sheets.

Gingerbread

Yield: Four 8" x 8" pans

Temp.: 375° F

Time : 20 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Flour	492	363.1	62.8	4.9
Baking Powder	15	---	---	---
Salt	5	---	---	---
Molasses	517	336.0	---	---
Sugar	170	170.0	---	---
Shortening	110	---	---	110.0
Soda	15	---	---	---
Ginger	2	---	---	---
Cinnamon	7	---	---	---
Cloves	2	---	---	---
Allspice	8	---	---	---
Boiling Water	400	---	---	---
Prepared Weight:	1504	869.2	62.8	114.9
Serving: (one)	100	57.8	4.2	7.6

Instructions: Line four 8" x 8" pans with wax paper. Combine flour with baking powder and salt. Mix sugar and molasses in electric mixing bowl. Add shortening and soda to sugar and molasses. Add flour mixture. Mix spices and boiling water. Add this spice mixture to the previously mixed ingredients. Pour 435 grams in each pan.

Harvard Beets

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Sugar	270	270.0	---	---
Cornstarch	14	10.5	---	---
Vinegar	145	---	---	---
Water	145	---	---	---
Beets (drain)	1200	117.1	11.6	1.4
Butter	75	0.6	0.4	60.4
Salt	3	---	---	---
Pepper	1	---	---	---
Prepared Weight:	1663	398.2	12.0	61.8
Serving: (one)	100	24.0	0.7	3.7

Instructions: Mix sugar and cornstarch in 3-quart sauce pan. Add vinegar and water. Boil 5 minutes. Add beets. Let simmer 30 minutes. Add butter, salt and pepper. Remove from heat and place in a pan of hot water to keep warm for serving.

Lemon Pie with Meringue

Yield: Two 9" pies

Ingredient	Gram Weight	CHO gm	Protein gm.	Fat gm.
Cornstarch	80	70.0	---	---
Flour	60	44.9	7.7	0.6
Salt	6	---	---	---
Sugar	740	740.0	---	---
Boiling Water	900	---	---	---
Butter	35	0.4	0.3	28.5
Lemon Juice (Real Lemon)	205	15.8	0.8	0.4
Egg Yolks (slightly beaten)	170	1.0	28.0	54.0
Meringue:				
Egg Whites	155	1.0	16.5	---
Sugar	120	120.0	---	---
Vanilla	6	---	---	---
Pie Crust	421	162.4	28.1	137.2
Prepared Weight: (1 pie)	1200	577.7	40.7	110.4
Serving (one) :	160	77.0	5.4	14.7

Instructions: Mix cornstarch, flour, salt and sugar. Add boiling water. Stir constantly over heat until the mixture boils. Cook until thickened. Add butter and lemon juice. Add half of the hot mixture to slightly beaten egg yolks. Mix. Add this egg mixture to the remaining hot mixture. Cook and stir until thick. Cool.

For the meringue beat the egg whites until stiff, then gradually beat in sugar until meringue is stiff and shiny. Add vanilla. Place 940 grams filling into 9" baked pastry shell (see Pie Crust). Top with 125 grams meringue. Bake at 350°F for 12 minutes.

Reference: Farmer, F. M. 1951. The Boston Cooking-School Cook Book 9th ed. Little Brown and Co., Boston. p. 637.

Lemon Sauce

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Sugar	200	200.0	---	---
Flour	28	21.4	2.8	0.3
Water	480	---	---	---
Butter	55	---	---	44.6
Lemon Juice	15	1.2	0.1	---
Nutmeg	5	---	---	---
Prepared Weight:	720	222.6	2.9	44.9
Serving: (one)	50	15.8	0.2	3.2

Instructions: Combine sugar and water in 2-quart sauce pan. Stir in flour to make a paste. Bring to a boil. Remove from heat. Stir in butter, lemon juice, and nutmeg.

Lime Sherbet[1]

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Milk (3.5% Fat)	19,505	991.2	719.4	679.4
Water	12,928	---	---	---
Sugar- (cane)	9,072	9072.0	---	---
Sugar- (corn)	3,629	3276.5	---	---
Stabilizer	226.8	---	---	---
Prepared Weight:	45,361	13339.7	719.4	679.4
Serving: (one)	75	22.6	1.2	1.1

[1] Prepared at the Michigan State University Dairy Department.
Calculation based on information obtained from the Dairy Department.

Macaroni Salad

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Macaroni (Dry) (Wet)	1200 2100	634.5	106.5	12.0
Onion (raw, chopped)	80	8.3	1.1	0.2
Celery (raw, diced)	320	11.8	4.2	0.6
Lettuce (torn)	200	5.8	2.4	0.4
Pickle Relish	120	31.4	0.9	0.9
Pimento (minced)	30	1.4	0.2	0.1
Celery seed	21	---	---	---
Dressing:				
Salad Oil	220	---	---	220.0
Vinegar	240	---	---	---
Water	120	---	---	---
Dry Mustard	1	---	---	---
Celery Salt	2	---	---	---
Black Pepper	dash	---	---	---
Paprika	2	---	---	---
Caraway Seed	4	---	---	---
Salt	5	---	---	---
Sugar	15	15.0	---	---
Thyme	pinch	---	---	---
Prepared Weight:	3490	708.2	115.3	234.2
Serving (one) :	240	48.7	7.9	16.1

Instructions: Cook macaroni in 7 quarts salted (2 Tbsp.) boiling water for 15 minutes. Drain. Rinse thoroughly with hot water. Drain. Add remaining ingredients. Mix. Cover and marinate 1 hour in refrigerator.

Mashed Potatoes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Potatoes (cooked, well drained)	1800	343.8	36.0	1.8
Butter	50	0.4	0.3	40.2
Milk	100	5.1	3.7	3.5
Salt	12	---	---	---
Pepper	1	---	---	---
Prepared Weight:	1950	349.3	40.0	45.5
Serving (one):	100	18.3	2.1	2.4

Instructions: Peel potatoes. Cut in halves. Cover with water and cook 30 minutes. Remove and drain. Mash potatoes with the use of the electric mixer. Combine milk and butter in 1 quart sauce pan. HEAT. Do not scald or boil. Add the milk mixture and the seasonings to the potatoes. Mix at low speed for 1 minute, then at high speed until light and fluffy. Place the mashed potatoes in a container. Place container in hot water until all the subjects are served.

Meats

The protein and fat composition of the meat prepared was obtained from the tables of food composition.

Beef (choice grade, bottom, round roast): Season with salt and pepper. Place with fat side up in roaster. Bake at 325°F. Allow 40 minutes for each pound of roast. Trim off fat and serve.

Beef Patties (choice grade, ground beef): Form 75 grams of ground beef into a patty. Cook in electric frying pan. When done place on paper towels to absorb fat.

Canadian Bacon (roll): Trim and slice into 50 gram portions. Place on baking sheet. Warm in oven at 200°F for 30 minutes.

Chicken (roasting): Season with salt and pepper. Place in roaster and bake at 325°F allowing 40 minutes per pound of chicken. Prior to serving, remove fat and bones. Serve a combination of white and dark meat to each subject.

Cube Steak (choice grade): Season with salt and pepper. Pan broil. When done place on paper towels to absorb any excess fat.

Fish (frozen, White): Thaw. Weigh 65 gram portions and place on baking sheet. Top each portion with 5 grams butter. Season with salt and pepper. Bake at 325°F for 45 minutes.

Ham (canned): Trim excess fat, slice and weigh individual servings.

Pork Chops (choice grade, center cut): Trim fat. Season with salt and pepper. Place on baking sheet. Bake at 325°F for 50 minutes.

Veal cutlets (choice grade): Season with salt and pepper. Place on baking sheet. Bake at 325°F for 40 minutes.

Molasses Cookies

Temp.: 350°F

Time : 10 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Shortening	200	---	---	200.0
Brown Sugar	220	210.0	---	---
Hot Water	240	---	---	---
Molasses (medium)	320	192.0	---	---
Flour	500	369.0	63.8	5.0
Salt	5	----	---	---
Soda	2	---	---	---
Baking Powder	8	---	---	---
Ginger	5	---	---	---
Cloves (ground)	3	---	---	---
Cinnamon	7	---	---	---
Grapenut Flakes	120	98.6	11.6	1.7
Prepared Weight:	1341	869.6	75.4	206.7
Serving (one) :	50	32.4	2.8	7.7

Instructions: Cream shortening and sugar in Hobart mixing bowl. Add hot water to molasses. Add the water and molasses mixture to the shortening and sugar. Combine dry ingredients and add, mixing at low speed for 2 minutes. Drop dough from teaspoon onto a lightly greased cookie sheet. Remove from baking sheet and place on wax paper. When cool, weigh and store in plastic bags in the refrigerated walk-in. Be sure to label the bag with the contents, weight and the date prepared.

Oatmeal

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Oatmeal (dry weight)	600	409.2	85.2	44.4
Water	2900	---	---	---
Salt	10	---	---	---
Prepared Weight:	3100	409.2	85.2	44.4
Serving (one) :	236	31.2	6.5	3.4

Instructions: Stir oats into boiling salted water. Cook 5 minutes, stirring occasionally. Cover pan, remove from heat and place in a pan of hot water until all subjects are served.

Peach Crumb Pie

Yield: Two 9" pies

Temp.: 375°F

Time : 45 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Filling:				
Sugar	400	400.0	---	---
Flour	56[1]	---	---	---
Salt	2	---	---	---
Peaches(frozen, sliced, sweetened)	1000	226.2	4.1	0.8
Crust:				
Salt	5	---	---	---
Flour	220[1]	---	---	---
Shortening	135	---	---	135.0
Water	60	---	---	---
Topping:				
Flour	85[1]	---	---	---
Brown Sugar (light)	110	105.0	---	---
Butter (control)	56	0.4	0.3	45.1
(Exper. - protein free)	56	---	---	44.8
Baking Powder	4	---	---	---
[1] Combined values for flour	361	266.4	46.1	3.6
Prepared Weight: (Control)	1981	998.0	50.5	184.3
(Exper.)	1980	997.6	50.2	184.2
Serving (one): (Control)	135	68.0	3.4	12.6
(Exper.)	135	68.0	3.4	12.6

Instructions: Drain peaches. Combine dry filling ingredients. Mix thoroughly with peaches. For preparation of crust, see Pie Crust. Fill each crust with 715 grams of filling. Blend topping ingredients and sprinkle 125 grams over the filling of each pie.

Pettijohn Cereal

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Pettijohn (dry)	448	339.2	41.6	9.6
Water	2432	---	---	---
Salt	8	---	---	---
Prepared Weight:	2500	339.2	41.6	9.6
Serving (one):	180	24.2	3.0	0.7

Instructions: Bring salted water to boil. Sprinkle in pettijohn. Cook 5 minutes stirring occasionally. Remove from heat. Cover and place in receptacle of hot water to keep warm until all subjects are served.

Pie Crust

Yield: Two double crust pies

Temp.: 400°F

Time : 12 minutes

Ingredients	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Salt	12	---	---	---
Flour	440	324.8	56.1	4.4
Shortening	270	---	---	270.0
Water (cold)	120	---	---	---
Prepared Weight: (raw weight)	842	324.8	56.1	274.4

Serving (one): Calculated with each total pie recipe

Instructions: Cut shortening into flour, to which salt has been added, with a pastry blender. Add cold water. Mix lightly with a fork. Roll on wax paper.[1] For double crust, weigh out 421 grams per pie. For single crust, use 210 grams per pie. Bake at 400°F for 12 minutes.[2]

[1] NO extra flour is to be used in rolling the crust.
[2] Only bake shell when required by recipe.

Pineapple Upside Down Cake

Yield: Three 8" x 8" cakes

Temp.: 350°F

Time : 30 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Butter (protein free)	70	---	---	56.0
Brown Sugar	275	262.6	---	---
Pineapple slices (drained)[1]	488	184.1	3.6	0.7
Flour	338	249.5	43.1	3.4
Sugar	250	250.0	---	---
Baking Powder	20	---	---	---
Salt	7	---	---	---
Shortening	163	---	---	163.0
Pineapple juice[1]	385	---	---	---
Prepared Weight:	1715	946.2	46.7	223.1
Serving (one):	75	41.4	2.0	9.8

Instructions: Melt butter in 1 quart sauce pan. Add brown sugar. Place 175 grams of the sugar and butter mixture in each pan. Arrange 162 grams of drained pineapple in this mixture. Combine dry ingredients and soften shortening in electric mixer bowl. Add pineapple juice. Beat 2 minutes. Weigh and divide into thirds (approximately 540 grams). Place this amount in each pan. When done, let stand 5 minutes. Then invert on wax paper.

[1] Pineapple calculated values represent 488 grams of slices and 385 grams of juice.

Pizza Dough

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Sugar	30	30.0	---	---
Salt	16	---	---	---
Shortening	35	---	---	35.0
Boiling Water	300	---	---	---
Lukewarm Water	450	---	---	---
Yeast (dry)	18	7.0	6.6	0.3
Flour	1130	834.1	144.2	11.3
Prepared Weight:	1979	871.1	150.8	46.6
Serving (one) : (wet weight)	150	65.5	11.3	3.5

Instructions: Combine yeast with 150 grams of lukewarm water in a 1 quart mixing bowl. Set aside in warm area. Place salt, shortening and boiling water in Hobart mixing bowl. Beat until shortening is dissolved. Add 300 grams lukewarm water. Add sugar and yeast mixture. Add three cups of flour and beat until smooth. Add remaining flour. Knead at low speed for 5 minutes. Into each 8" aluminum foil pie plate, place 150 grams dough. Flatten the dough so that it covers the bottom of the pan and comes up on the sides 1/2 inch.

Pizza Sauce

Temp.: 425°F

Time : 20 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Canned tomatoes (drained)	2760	107.6	27.6	5.5
Onion (fresh, chopped)	130	13.4	1.8	0.3
Green Pepper (fresh, chopped)	325	18.6	3.9	0.9
Sweet Basil	25	---	---	---
Oregano	5	---	----	---
Salt	5	---	---	---
Bouillon Cubes (2 vegetable cubes)	8	---	1.0	---
Protein free butter	28	---	---	22.4
Prepared Weight:	2994	139.6	34.3	29.1
Serving (one) :	175	8.2	2.0	1.7

Instructions: Combine all ingredients in an 8 quart aluminum kettle. Cook 45 minutes. Cover each individual serving of pizza dough with 150 grams of sauce. Bake at 425°F for 20 minutes. After baking, turn oven off -- leave pizzas in oven until all subjects are served.

Potato Salad

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Cold boiled potatoes (peeled)	900	171.9	18.0	0.9
Onion (raw, chopped)	40	4.1	0.6	0.1
Parsley (dry)	7	0.1	0.1	---
Pickle Relish	50	13.1	0.4	0.4
Vinegar	20	---	---	---
Salad Oil	85	---	---	85.0
Salt	8	---	---	---
Prepared Weight:	1110	189.2	19.1	86.4
Serving (one) :	75	12.8	1.3	5.8

Instructions: Scrub potatoes with skins and place in 8 quart kettle. Cover with water and cook 45 minutes. Drain. Peel and slice potatoes into large bowl. Add onions, parsley, and pickle relish. Combine vinegar, salad oil and salt. Pour this mixture over the potatoes. Toss lightly. Keep in cool place until time of serving. Turn once before serving.

Potatoes au Gratin

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Potatoes (cooked)	1400	267.4	28.0	1.4
Butter	75	0.6	0.5	60.4
Flour	40	29.5	5.2	0.4
Milk	1350	68.6	49.0	49.8
Salt	8	---	---	---
Pepper	dash	---	---	---
Parsley (dried)	5	0.3	0.1	0.1
Prepared Weight:	2650	366.4	82.8	112.1
Serving (one) :	130	18.2	4.0	5.2

Instructions: Peel 2000 grams raw potatoes. Cook until done. Melt butter in 8 quart aluminum kettle. Stir flour into butter. Add milk gradually. Simmer 15 minutes. Add salt and pepper. Add potatoes. Then add parsley. Place 130 grams potato mixture into a casserole. Top with 20 grams bread crumbs and 5 grams butter. Place in warming oven until all subjects are served.

Refrigerator Cookies (Control Period)

Temp.: 350° F

Time : 8 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Butter	670[1]	5.4	4.0	539.4
Sugar	300	300.0	---	---
Flour	825	608.9	105.3	8.2
Lemon or Vanilla Extract	20	---	---	---
Prepared Weight:	1571	914.3	109.3	547.6
Serving (one) :	50	29.1	3.5	17.4

Instructions: Cream sugar and butter until fluffy in Hobart mixing bowl. Add flour and flavoring. Mix at low speed for 3 minutes. Form oblong roll and wrap in wax paper. Chill in refrigerator. Slice crosswise. Bake on lightly greased baking sheet. Remove from baking sheet and place on wax paper. When cool, weigh and store in plastic bag in the refrigerated walk-in. Be sure to label bag with the contents, weight and the date prepared.

Reference: Swedish Food, 1946, 5th ed. Esselte, Gothenburg, Sweden. p. 132.

[1] 10 grams of butter is to be used to grease the cookie baking sheets.

Refrigerator Cookies (Experimental Period)

Temp.: 350°F

Time : 8 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Butter (protein free)	670 [1]	---	---	536.0
Sugar	300	300.0	---	---
Flour	825	608.9	105.3	8.2
Lemon Extract (or Vanilla Extract	20	---	---	---
Prepared Weight:	1570	908.9	105.3	544.2
Serving (one) :	50	29.0	3.4	17.3

Instructions: Cream sugar and butter until fluffy in Hobart mixing bowl. Add flour and flavoring. Mix at low speed for 3 minutes. Form oblong roll and wrap in wax paper. Chill in refrigerator. Slice crosswise. Bake on lightly greased baking sheet. Remove from baking sheet and place on wax paper. When cool, weigh and store in plastic bag in the refrigerated walk-in. Be sure to label bag with the contents, weight and the date prepared.

[1] 10 grams of butter is to be used to grease the cookie baking sheet.

Rice (Boiled)

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Rice (dry)	418	332.2	31.9	1.1
Water	1922	---	---	---
Salt	10	---	---	---
Prepared Weight:	1300	332.2	31.9	1.1
Serving (one) :	100	26.2	2.5	0.1

Instructions: Bring water and salt to boil. Add rice and stir. Cook for 30 minutes using medium heat. Remove cover, place container in pan of hot water to keep warm until all subjects are served.

Shortcake

Temp.: 400°F

Time : 15 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Flour	550	406.0	70.2	5.5
Sugar	60	60.0	---	---
Baking Powder	30	---	---	---
Salt	20	---	---	---
Shortening	165	---	---	165.0
Water	505	---	---	---
Prepared Weight: (raw weight)	1330	466.0	70.2	170.5
Serving (one): (raw weight)	65	22.8	3.4	8.3

Instructions: Combine dry ingredients. Cut in shortening with a pastry blender. Add water. Stir only to mix. Weigh out 65 grams raw weight per subject. Bake on ungreased baking sheet.

Shredded Cabbage with Pineapple Salad

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Cabbage (shredded)	420	22.7	5.9	0.8
Pineapple Slices (canned)	280	59.0	1.2	0.2
Prepared Weight:	700	81.7	7.1	1.0
Serving (one) :	50	2.7	0.7	0.1

Instructions: Drain pineapple and cut into chunks. Shred cabbage. Combine these two and toss lightly. Serve 50 grams to each subject.

Spaghetti

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Control				
Spaghetti (enriched, dry weight)	530	407.9	68.1	7.4
Water	1580	---	---	---
Salt	10	---	---	---
Experimental				
Spaghetti (enriched, dry weight)	800	611.9	102.1	11.1
Water	2370	---	---	---
Salt	15	---	---	---
Prepared Weight: (Control)	1350	407.9	68.1	7.4
(Exper.)	2025	611.9	102.1	11.1
Serving (one): (Control)	100	30.2	5.1	0.6
(Exper.)	146	44.1	7.4	0.9

Instructions: Place water and salt in 8 quart aluminum kettle. Bring to boil. Add spaghetti. Cook 20 minutes at high speed. Drain. Rinse with hot water. Place the amount specified for the individual serving in the casserole dishes.

Spaghetti Sauce

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Onion (raw, diced)	250	25.8	3.5	0.5
Green Pepper (raw, diced)	250	14.3	3.0	0.7
Vegetable Oil	70	---	---	70.0
Salt	10	---	---	---
Sugar	9	9.0	---	---
Tomatoes (canned)	2400	93.6	24.0	4.8
Prepared Weight:	3000	142.7	30.5	76.0
Serving (one) :	200	9.6	2.0	5.0

Instructions: Sautee onion and green pepper in vegetable oil in 8 quart aluminum kettle. Add seasonings. Add tomatoes and heat thoroughly (30 minutes). Must total 3000 grams. Cover 146 grams of cooked spaghetti with 200 grams of this sauce in individual casserole. Place in oven prior to serving.

Spanish Rice

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Bacon, raw	84	0.6	6.9	48.6
Onion (raw, chopped)	600	61.8	8.4	1.2
Green Pepper (raw, chopped)	180	10.3	2.2	0.5
Tomato Soup (Condensed)	2700	329.8	43.5	54.4
Rice (dry weight)	450	357.6	34.3	1.2
Water	675	---	---	---
Whole Cloves	18	---	---	---
Bay leaves	4	---	---	---
Salt	12	---	---	---
Prepared Weight:	4550	760.1	95.3	105.9
Serving (one) :	300	50.1	6.3	7.0

Instructions: Cut bacon into small pieces. Fry until crisp in 8 quart aluminum kettle. Add onion and green pepper. Fry until golden. Add remaining ingredients. Cover and cook slowly 50 minutes. Remove cloves and bay leaves. Serve each subject 300 grams in individual casserole. Place casseroles in warming oven until all subjects are served.

Sweet Rolls

Temp.: 350° F

Time : 35 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Control				
Bread Dough	1700	772.2	131.3	51.8
Sugar	200	200.0	---	---
Butter	200[1]	1.6	1.2	161.0
Cinnamon	12	---	---	---
Prepared Weight:	1807	973.8	132.5	212.8
Serving (one) :	130	70.1	9.5	15.3
Experimental				
Bread Dough	3000	1363.0	231.6	91.5
Sugar	350	350.0	---	---
Protein free Butter	350[1]	---	---	280.0
Cinnamon	22	---	---	---
Prepared Weight:	3186	1713.0	231.6	371.5
Serving (one) :	200	107.5	14.5	23.3

Instructions: Roll dough to 1/2 inch thickness. Combine butter, sugar and cinnamon. Spread evenly over rolled out dough. Roll dough into a roll. Slice 1/2 inch thick and place on greased baking sheet. Cover and let rise one hour.

[1] 15 grams of the weighed butter for the Control sweet rolls is to be used to grease the baking sheet.
30 grams of the weighed butter for the experimental sweet rolls is to be used to grease the baking sheet.

Tomato and Rice Casserole

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Rice (converted, dry weight)	260	206.6	19.8	0.7
Salt	7	---	---	---
Water	810	---	---	---
Onions, raw (minced)	160	16.5	2.2	0.3
Celery, raw (diced)	160	5.9	2.1	0.3
Green Pepper, raw (diced)	160	9.2	1.9	0.4
Vegetable Shortening	50	---	---	50.0
Tomatoes (canned)	2000	78.0	20.0	4.0
Celery Salt	14	---	---	---
Sugar	8	8.0	---	---
Salt	28	---	---	---
Pepper	dash	---	---	---
Prepared Weight:	3555	324.2	46.0	55.7
Serving (one) :	245	22.3	3.2	3.8

Instructions: Cook rice in boiling salted water until tender. Cook onions, celery, green pepper in vegetable shortening in 8 quart aluminum kettle. Add tomatoes and remaining seasonings. Add cooked rice to this mixture. Serve 245 grams of tomato/rice in individual casseroles. This is to be topped with 25 grams of bread crumbs and 5 grams butter (protein free). Place casseroles in warming oven until all subjects are served.

Vegetable Chop Suey

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Vegetable Bouillon Cubes (2)	8	---	1.0	---
Water	3760	---	---	---
Flour	240	177.1	30.6	2.4
Salt	10	---	----	---
Pepper	1	---	---	---
Worcestershire Sauce	40	7.2	0.8	---
Soy Sauce	40	4.0	trace	0.5
Celery (fresh, diced)	1000	37.0	13.0	2.0
Onions (fresh, diced)	240	24.7	3.4	0.5
Soy Bean Sprouts (canned, drained)	135	11.8	11.8	1.7
Prepared Weight:	4884	261.8	60.6	7.1
Serving (one) :	350	18.8	4.3	0.5

Instructions: Combine bouillon cubes and 3000 grams of water in an 8 quart aluminum kettle. Bring to boil. Make smooth paste from 760 grams of water and flour. Add this paste to the bouillon water. Add remaining seasonings. Cook celery and onions in small amount of water. Drain and add to previous mixture. Add bean sprouts. Check total weight which should be 4884 grams.[1] Serve 350 grams of vegetable chop suey in each casserole. Place casseroles in oven to keep warm.

[1] Add water if required to meet this weight.

Vegetable Soup

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Potatoes (raw, diced)	300	57.3	6.0	0.3
Carrots (canned, drained)	725	46.4	4.8	3.9
Celery (raw, diced)	350	13.0	4.6	0.7
Butter (protein free)	50	---	---	40.0
Flour	40	29.5	5.1	0.4
Vegetable Bouillon Cubes (5)	20	---	2.5	---
Boiling Water	2700	---	---	---
Salt	9	---	---	---
Pepper	1	---	---	---
Prepared Weight:	3724	146.2	23.0	45.3
Serving (one) :	250	9.8	1.5	3.0

Instructions: Cook celery and potatoes in small amount of water until done. Drain. Melt butter in 8 quart aluminum kettle. Stir in flour until well blended. Slowly add boiling water. Add vegetable bouillon cubes. Add salt and pepper. Add vegetables. Cook slowly 10 minutes stirring constantly. Place 250 grams in each casserole, cover, and place in warming oven.

Vegetarian Stew

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Carrots (canned, drained, diced)	370	23.7	2.5	2.0
Potatoes (raw, diced)	180	34.4	3.6	0.2
Celery (raw, diced)	150	5.5	1.9	0.3
Wax Beans (canned, drained)	150	11.6	3.6	0.3
Water	960	---	---	---
Vegetable Bouillon Cubes (2)	8	---	1.0	---
Flour	40	29.5	5.1	0.4
Salt	3	---	---	---
Pepper	dash	---	---	---
Butter (protein free)	50	---	---	40.0
Prepared Weight:	975	104.7	17.7	43.2
Serving (one) :	75	8.0	1.4	3.3

Instructions: Cook potatoes and celery. Drain well. Melt butter in 8 quart aluminum kettle. Stir flour into it. Add hot water, vegetable bouillon cubes and seasonings. Add all the vegetables to this thickened sauce. Add dumplings (See dumpling recipe). Cook 20 minutes. Serve one dumpling and 75 grams vegetable stew in individual casseroles.

Wheatena

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Wheatena	672	520.8	69.6	14.4
Water	3648	---	---	---
Salt	8	---	---	---
Prepared Weight:	3780	520.8	69.6	14.4
Serving (one):	270	37.2	5.0	1.0

Instructions: Bring water to a boil. Add salt. Sprinkle in Wheatena slowly, so water never stops boiling. Cook 10 minutes. Remove from heat. Place receptacle in pan of hot water to keep warm for serving.

White Cake

Yield: Three 8" x 8" cakes

Temp.: 325°F

Time : 30 minutes

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Eggs	166	0.9	21.2	16.9
Sugar	525	525.0	---	---
Butter	196	1.6	1.2	157.8
Milk	420	21.3	15.5	14.6
Salt	4	---	---	---
Flour	490	361.7	62.5	4.9
Baking Powder	24	---	---	---
Vanilla	8	---	---	---
Prepared Weight:	1585	910.5	100.4	194.2
Serving (one) :	100	57.4	6.3	12.2

Instructions: Line three 8"x 8" cake pans with wax paper. Combine all dry ingredients in electric mixing bowl. Add the softened butter, vanilla, and two tablespoons of the milk. Beat 1 minute at medium speed. Add egg and remaining milk. Beat 3 minutes at high speed. Place 611 grams in each cake pan.

Reference: Farmer, F. M. 1951. The Boston Cooking School Cook Book 9th ed. Little Brown and Co., Boston, p. 701.

White Icing (for cupcakes)

Ingredient	Gram Weight	CHO gm.	Protein gm.	Fat gm.
Butter	75	0.6	0.4	60.4
Vanilla	3	---	---	---
Confectioner's sugar	340	338.3	---	---
Prepared Weight:	415	338.9	0.4	60.4
Serving (one) :	30	24.5	---	4.5

Instructions: Melt butter in 1 quart sauce pan. Remove from heat. Beat in vanilla and sugar. Frost cupcakes using 30 grams per subject.